A Dissertation on

HOMOCYSTEINE:
ASSOCIATION WITH PREECLAMPSIA AND ITS SEVERITY AND CORRELATION WITH MATERNAL AND FETAL OUTCOME OF PREECLAMPTIC WOMEN

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BONAFIDE CERTIFICATE

Certified that this dissertation is the bonafide work of Dr. S.ANURADHA on **HOMOCYSTEINE: ASSOCIATION WITH PREECLAMPSIA AND ITS SEVERITY AND CORRELATION WITH MATERNAL AND FETAL OUTCOME OF PREECLAMPTIC WOMEN** during her M.D.,(Obstetrics & Gynaecology) course from April 2009 to April 2012 at the Stanley Medical College and Raja Sir Ramasamy Mudaliar Lying-in Hospital, Chennai.

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DECLARATION

I solemnly declare that the dissertation titled "**HOMOCYSTEINE: ASSOCIATION WITH PREECLAMPSIA AND ITS SEVERITY AND CORRELATION WITH MATERNAL AND FETAL OUTCOME OF PREECLAMPTIC WOMEN"** is done by me at RSRM Lying in Hospital, Stanley Medical College and Hospital, Chennai during September 2010 to September 2011 under the guidance and supervision of **Prof.D.Tamilselvi M.D.,O.G,** Professor & Chief of the department of Obstetrics and Gynaecology, Stanley Medical College & RSRM Lying In Hospital, Chennai-13.

This dissertation is submitted to the, The Tamilnadu Dr.M.G.R Medical University towards the partial fulfillment of requirements for the award of M.D.Degree (Branch-2) in Obstetrics and Gynaecology.

Place: DR.S.ANURADHA

Date:

ABBREVIATIONS

APGAR	-	**A**ppearance, **P**ulse, **G**rimace, **A**ctivity, **R**espiration
DIC	-	Disseminated Intravascular Coagulation
HELLP	-	Hemolysis, Elevated Liver enzymes, Low Platelets
HLA-G	-	Human Leucocyte Antigen G
IUGR	-	Intra Uterine Growth Restriction
IUD	-	Intra Uterine Death
MGA	-	Maternal Gestational Age
NICU	-	Neonatal Intensive Care Unit
PLGF	-	Placental Like Growth factor
PPH	-	Post Partum Hemorrhage
SAM	-	S-Adenosyl Methionine
sENG	-	soluble Endoglin
sFlt-1	-	soluble Fms-like tyrosine kinase 1
THFA	-	Tetra Hydro Folic Acid
VEGF	-	Vascular Endothelial Growth Factor

INTRODUCTION

Preeclampsia is a pregnancy specific disorder which complicates about 3-10% of all nulliparous gestations[1]. The incidence is markedly influenced by race and ethnicity. Preeclampsia is the most common serious medical disorder of human pregnancy. It is characterized by the development of hypertension with proteinuria after 20 weeks of gestation. Edema is often present but is not a requisite for the diagnosis. Preeclampsia is still regarded as a disease of theories and its etiology has remained poorly understood. Although the specific cause still remains unknown, endothelial dysfunction has been considered central in the pathophysiology of preeclampsia.

Homocysteine is a sulfur containing aminoacid primarily derived from the demethylation of dietary methionine required for the growth of cells and tissues in the human body. Hyperhomocysteinemia leads to endothelial dysfunction through various mechanisms. Homocysteine also interferes with the fibrinolytic system adding to the pathophysiology of preeclampsia.

In normal pregnancy homocysteine levels are decreased than the non pregnant level[2], either due to hemodilution of pregnancy or the relative deficiency due to increase in requirement by the mother and fetus.

Maternal hyperhomocysteinemia has been associated with a number of pregnancy related diseases like preeclampsia, placental abruption, recurrent pregnancy loss, still birth, deep vein thrombosis and neural tube defects in the newborn[3].

It has been proposed that hyperhomocysteinemia may be associated with preeclampsia as the homocysteine mediated vascular damages are similar to those associated with preeclampsia. Though many studies are done elsewhere, studies on homocysteine in pregnancy are limited in South India. Also there are limited studies correlating maternal homocysteine level with the severity of preeclampsia and with the maternal and fetal outcomes of preeclamptic mothers.

This study is mainly aimed to find the association between maternal homocysteine levels and preeclampsia and its severity and the correlation of maternal homocysteine level with maternal and fetal outcome of preeclamptic mothers .

AIMS AND OBJECTIVES OF THE STUDY

- To study the association of homocysteine levels among normal, mild and severe preeclamptic pregnant women.
- To study the association between the maternal homocysteine levels and the severity of preeclampsia.
- Maternal outcome including maternal morbidities due to the various complications of preeclampsia are to be studied for any association with the homocysteine level.
- Foetal outcome like term/preterm , IUD, IUGR, neonatal mortality, birth weight ,APGAR score, duration of NICU stay are correlated with the maternal homocysteine level to find out any significant association.

REVIEW OF LITERATURE

PREECLAMPSIA

Preeclampsia is a pregnancy specific syndrome which is characterized by variable degrees of placental dysfunction and a maternal response featuring systemic inflammation. Hypertension and proteinuria are considered to be the hallmark's of preeclampsia[4-6] but the clinical manifestations of this syndrome are highly heterogeneous. The clinical findings of preeclampsia can manifest as either a maternal syndrome (hypertension and proteinuria with or without other multisystem abnormalities) or as a fetal syndrome (fetal growth restriction, reduced amniotic fluid, and abnormal oxygenation)[4,5].

DEFINITION OF PREECLAMPSIA:

The classic triad of preeclampsia includes hypertension, proteinuria and edema. However, there is now universal agreement that edema should not be considered as part of the diagnosis of preeclampsia.[7–13] Indeed, edema is neither sufficient nor necessary to confirm the diagnosis of preeclampsia, because edema is a common finding in normal pregnancy and approximately one third of eclamptic women never demonstrate edema.[14]In general, preeclampsia is primarily defined as hypertension occurring after 20 weeks of gestation together with proteinuria.

Hypertension in pregnancy is defined as

- systolic blood pressure more than or equal to 140mm of Hg
- diastolic blood pressure greater than or equal to 90 mm of Hg

- these measurements must be confirmed by repeated readings over 4 to 6 hours[15,16].

The gold standard for determining proteinuria is the 24-hour urinary protein excretion. Proteinuria is defined as

- Excretion of more than 300mg of protein/24hr
- Or a urinary protein-to-creatinine ratio of 30mg/mmol
- Or if the above methods are unavailable, as a concentration of 30mg/dL($\geq$ 1+ on dipstick) or more in at least 2 random urine samples collected at least 4 to 6 hours apart.
- Urinary tract infection must be excluded before attributing the proteinuria to preeclampsia.

Dipstick testing should be used only for screening purposes where other methods are not available. In all pregnant women who present with gestational hypertension the presence of proteinuria must be confirmed with a protein-to- creatinine ratio[15,16] or, preferably, a 24-hour urine collection.

The traditional criteria to confirm a diagnosis of preeclampsia (new onset of hypertension and proteinuria after 20 weeks' gestation) are appropriate to use in the majority of healthy nulliparous women. But, hypertension or proteinuria may be absent in 10 to 15 percent of women who develop the syndrome of hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome),[18] and in 20 to 25 percent of those who develop eclampsia.[19] In recognition that severe disease may occur in the absence of proteinuria, the Society for Obstetric Medicine Australia and New Zealand(SOMANZ) has widened these criteria to include the presence of other systemic manifestations, whether or not proteinuria is present (table-1).[20]

TABLE -1
SOCIETY FOR OBSTETRIC MEDICINE AUSTRALIA AND NEW ZEALAND DEFINITION OF PREECLAMPSIA
A clinical diagnosis of preeclampsia can be made when hypertension arises after 20wk' gestation and is accompanied by one or more of the following:
• **Renal involvement:** - Proteinuria ≥ 300mg/24 hr or a urine protein/creatinine ratio ≥ 30 mg/mmol - Serum or plasma creatinine ≥ 0.09mmol/L or oliguria (<500mL/24hr)
• **Hematological involvement:** - Thrombocytopenia - Disseminated intravascular coagulation - Hemolysis
• **Liver involvement:** - Abnormal liver function(AST and/or ALT >50 IU/L, raised bilirubin >25 IU/L) - And/or severe epigastric or right upper quadrant pain.
• **Neurologic involvement:** - Convulsions(eclampsia) - Hyperreflexia with sustained clonus - Severe headache(persisting, atypical) - Persistent visual disturbances (photopsia, scotomata, cortical blindness, retinal vasospasm) - Stroke
• **Pulmonary edema**
• **IUGR and/or signs of fetal distress**
• **Placental abruption**

CLASSIFICATION OF PREECLAMPSIA:

The American College of Obstetricians and gynaecologists classify preeclampsia dichotomously as non severe and severe.[21]

ABNORMALITY	MILD/NON SEVERE	SEVERE
Diastolic blood pressure	< 110mm of Hg	≥ 110mm of Hg
Systolic blood pressure	< 160mm of Hg	≥ 160mm of Hg
Proteinuria	≤ 2+	≥ 3+
Headache,visual disturbance	Absent	Present
Upperabdominal pain, oliguria	Absent	Present
Serum creatinine	Normal	Elevated
Thrombocytopenia/ pulmonary edema	Absent	Present
Transaminase elevation	Minimal	Marked
Fetal growth restriction	Absent	Obvious

PREECLAMPSIA SUPERIMPOSED ON CHRONIC HYPERTENSION:

This is diagnosed when:

A) There is a new-onset proteinuria ≥ 300mg/24 hours in hypertensive women but no proteinuria before 20 weeks of gestation.

B) A sudden increase in proteinuria or blood pressure or platelet count < 100,000/μL in women with hypertension and proteinuria before 20 weeks gestation[22].

INCIDENCE AND RISK FACTORS:

The incidence of preeclampsia ranges between 3 and 10 percent in healthy nulliparous women.[1,12] In these women, preeclampsia is generally mild, with the onset near term or intrapartum (75 percent of cases), and the condition conveys only a minimally increased risk for adverse pregnancy outcome.[7,9,23] In contrast, the severity of preeclampsia are substantially higher in women with multifetal gestation,[24,25] chronic hypertension,[23,26] previous preeclampsia,[23,27] pregestational diabetes mellitus,[23] and in those with preexisting thrombophilias.[28-30]

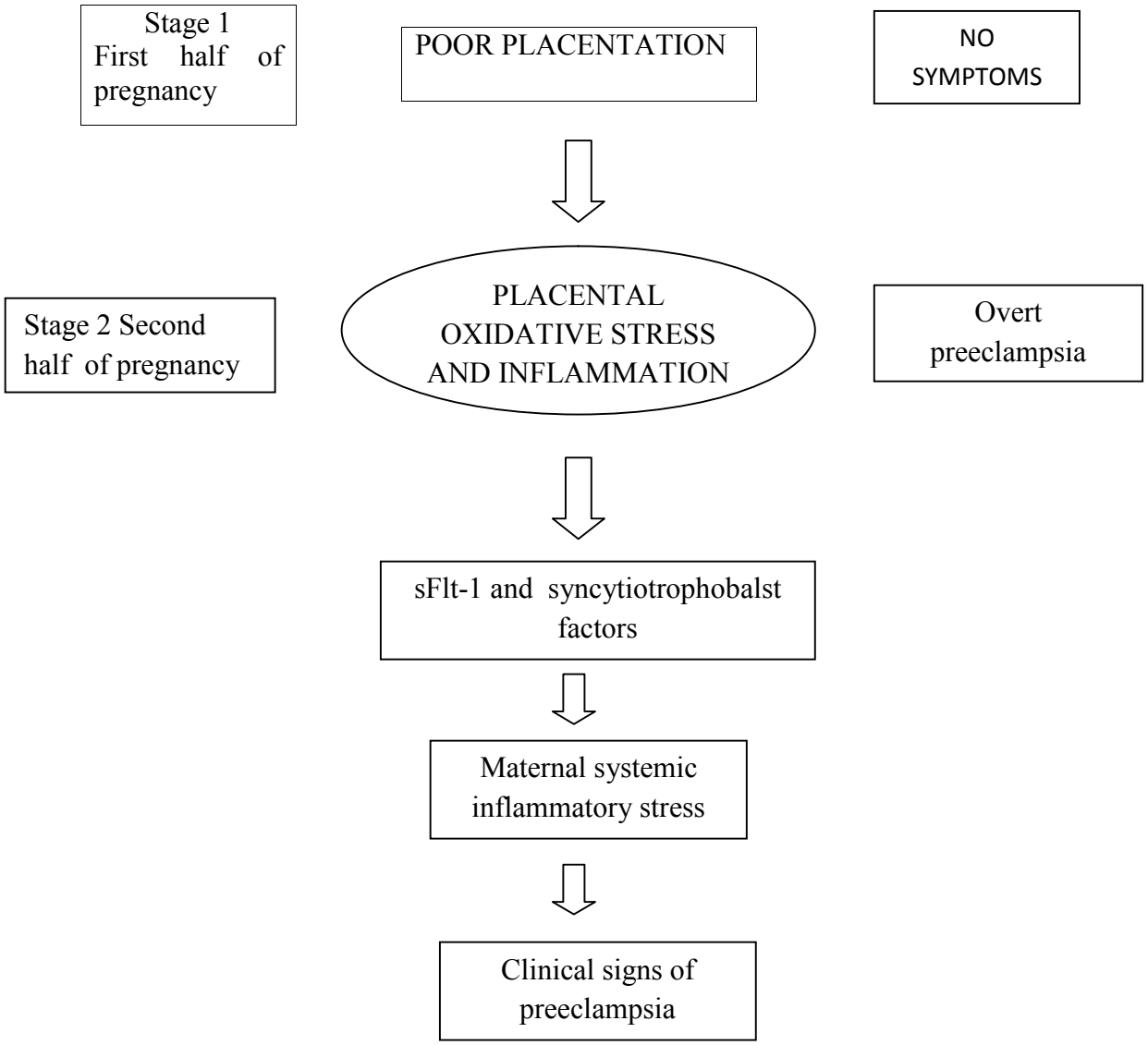
Generally, preeclampsia is considered a disease of primigravid women. The risk increases in those who have limited sperm exposure with the same partner before conception.[31-36] The recent advances in assisted reproductive technology have introduced several challenges for the maternal immune system that also increase the risk of preeclampsia.[25,31,37] The risk factors for preeclampsia are tabulated in table-2.

TABLE-2
RISK FACTORS FOR PREECLAMPSIA
Couple related risk factors: • Primipaternity and Limited sperm exposure • Pregnancy after artificial insemination • Protective effect of partner change in case of previous preeclamptic pregnancy
Maternal or pregnancy related risk factors: • Extremes of maternal age. • Multifetal gestation, preeclampsia in a previous pregnancy • Chronic hypertension and/or renal disease. • Maternal chronic inflammatory conditions(e.g., SLE) • Maternal chronic infections, Maternal low birth weight • Obesity and insulin resistance and Pregestational diabetes mellitus • Preexisting thrombophilias, Maternal susceptibility genes • Family history of preeclampsia, Hydropic degeneration of placenta • Smoking(reduced risk)

ETIOPATHOGENESIS OF PREECLAPMSIA:

The exact etiology of preeclampsia is still unknown. Redmann, Sargent and coworkers have proposed the two stage hypothesis for preeclampsia. According to this model, stage 1 is caused by faulty endovascular trophoblastic remodeling leading to poor placentation which causes the stage 2 clinical syndrome due to placental oxidative stress and inflammation. Stage 1 occurs in the first half of pregnancy and stage2 in the second half of pregnancy.

THE TWO STAGE MODEL OF PRE-ECLAMPSIA

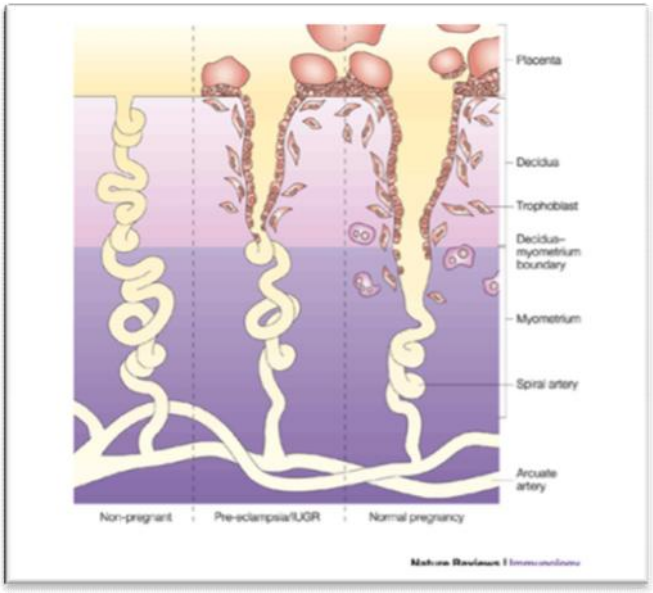


Such compartmentalization is arbitrary as preeclampsia is a continuous process. Preeclampsia is more likely a continuum of worsening disease.

UTERINE VASCULAR CHANGES:

The human placenta receives its blood supply from numerous uteroplacental arteries that are developed by the action of migratory interstitial and endovascular trophoblast into the walls of the spiral arterioles. This transforms the uteroplacental arterial bed into a low-resistance,low-pressure, high-flow system. The conversion of the spiral arterioles of the non pregnant uterus into the uteroplacental arteries has been termed *physiologic changes*.[38] In a normal pregnancy, these trophoblast-induced vascular changes extend all the way from the intervillous space to the origin of the spiral arterioles from the radial arteries in the inner one third of the myometrium. It is suggested that these vascular changes are effected in two stages: "the conversion of the decidual segments of the spiral arterioles by a wave of endovascular trophoblast migration in the first trimester and the myometrial segments by a subsequent wave in the second trimester."[38] These vascular changes result in the conversion of approximately 100 to 150 spiral arterioles into distended, tortuous, and funnel-shaped vessels that communicate through multiple openings into the intervillous space.

In contrast, pregnancies complicated by preeclampsia or by fetal growth restriction demonstrate inadequate maternal vascular response to placentation. In these pregnancies, the above-mentioned vascular changes are usually found only in the decidual segments of the utero placental arteries. Hence, the myometrial segments of the spiral arterioles are left with their musculo elastic architecture, thereby leaving them responsive to hormonal influences.[38] It is important to note that these vascular changes may also be demonstrated in a significant proportion of normotensive pregnancies complicated by fetal growth restriction.[38,39,41] Meekins and associates[40] have found that endovascular trophoblast invasion is not an all-or-none phenomenon in normal and preeclamptic pregnancies.



Uterine vascular changes in normal and preeclamptic pregnancy (FIG-1)

VASCULAR ENDOTHELIAL ACTIVATION AND PRO AND ANTIANGIOGENIC PROTEINS IN PREECLAMPSIA:

The endothelium is one of the key organs involved in the pathophysiology of preeclampsia, as evidenced by the prostacyclin, thromboxane imbalance[40-42] and impairment of nitric oxide synthesis which is a potent vasodilator. Endothelial activation leads to activation of platelets leading to spiral artery thrombosis thereby reducing the uteroplacental blood flow. In preeclampsia, endothelial cell dysfunction and platelet aggregation precede the increase in thrombin and fibrin formation.[43-46]

The mechanism by which placental ischemia leads to the clinical syndrome of preeclampsia is thought to be related to Soluble fms-like tyrosine kinase 1 (sFlt-1) , a protein that is produced by the placenta. It acts by binding to the receptor binding domains of vascular endothelial growth factor (VEGF), and it also binds to placental like growth factor (PLGF). Increased levels of this protein in the maternal circulation results in reduced levels of free VEGF and free PLGF, with resultant endothelial cell dysfunction.[42] Maynard et al.[47] demonstrated that soluble placenta-derived VEGF receptor (sFlt1), an antagonist of VEGF and PLGF, is unregulated in preeclampsia, leading to increased systemic levels of sFlt1

that fall after delivery. Increased sFlt1 in preeclampsia is associated with decreased circulating levels of free VEGF and PLGF, resulting in endothelial dysfunction. The magnitude of increase in sFlt levels correlates with disease severity[48,49,50] . Again, these data are compatible with decidual angiogenic growth factors, in particular PLGF, as being essential for early placental development with a later involvement of sFlt as a fetal rescue signal steering the maternal response, that is, the degree of maternal systemic hypertension. This hypothesis is supported by Levine et al.,[49,51] Recently soluble endoglin (sEng), a placental derived protein is found to increase months before clinical preeclampsia develops.[52] This soluble endoglin causes decreased endothelial nitric oxide-dependent vasodilatation thereby leading to maternal hypertension and endothelial dysfunction.

IMMUNOLOGY OF PREECLAMPSIA:

Loss of maternal immune tolerance to paternally derived placental and foetal antigens is another hypothesis cited for preeclampsia syndrome. Tolerance dysregulation might also explain an increased risk when the paternal antigenic load is increased, that is with two sets of paternal chromosomes- “a double dose” as occurring in molar preganancies and in trisomy 13.

HLA-G which is expressed on the cytotrophoblasts act as immunosuppresants thereby protecting the trophoblast from the cell mediated destruction of the uterine natural killer cells. In women who are prone to be preeclamptic the level of HLA-G was found to be low in the placenta thereby leading to recognition of the fetal cytotrophoblast as foreign.[53]

Contributors to an enhanced immunologically mediated inflammatory reaction are from the placental microparticles which are circulating the maternal body. These placental microparticles activate the maternal immune system leading to the maternal immune response syndrome which leads to oxidative injury of the placenta thereby the stage 2 of the two stage model hypothesis for preeclampsia. This lead to the formation of a new hypothesis called as the **placental debris hypothesis of preeclampsia.**[54]

GENETIC CONFLICT HYPOTHESIS:

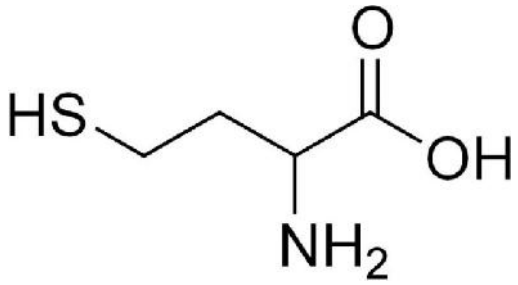
According to Haig's genetic conflict theory,[55] fetal genes are selected to increase the transfer of nutrients to the fetus, whereas maternal genes are selected to limit transfer in excess of some optimal level. The phenomenon of genomic imprinting means that a similar conflict exists within fetal cells between genes that are maternally derived and genes that

are paternally derived. The conflict hypothesis suggests that placental factors (fetal genes) act to increase maternal BP, whereas maternal factors act to reduce BP. [55,56] . Genome-wide linkage studies have identified at least three preeclampsia loci showing significant linkage: 2p12 , 2p25 and 9p13.[57] A direct proof of the role of imprinting was recently published by Oudejans et al.[58] Oudejans et al. confirmed the susceptibility locus on chromosome 10q22.1.

HOMOCYSTEINE

Homocysteine is a non essential sulfur containing aminoacid .

STRUCTURE OF HOMOCYSTEINE(FIG-2)



HOMOCYSTEINE METABOLISM:

Homocysteine is a non-protein–forming sulfur amino acid whose metabolism is at the intersection of two metabolic pathways: remethylation and transsulfuration.

1). In **remethylation**, homocysteine acquires a methyl group from N-5-methyltetrahydrofolate or from betaine to form methionine. The reaction

with N-5-methyltetrahydrofolate occurs in all tissues and is vitamin B12 dependent, whereas the reaction with betaine is confined mainly to the liver and is vitamin B12 independent. A considerable proportion of methionine is then activated by ATP to form *S*-adenosylmethionine (SAM). SAM serves primarily as a universal methyl donor to a variety of acceptors. *S*-adenosylhomocysteine (SAH), the by-product of these methylation reactions, is subsequently hydrolyzed, thus regenerating homocysteine, which then becomes available to start a new cycle of methyl-group transfer. (FIG-1)

2) In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by the pyridoxal-50-phosphate (PLP)–containing enzyme, cystathionine - synthase. Cystathionine sulfates is excreted in the urine. Thus, in addition to the synthesis of cysteine, this transsulfuration pathway effectively catabolizes excess homocysteine, which is not required for methyl transfer.(FIG-3).

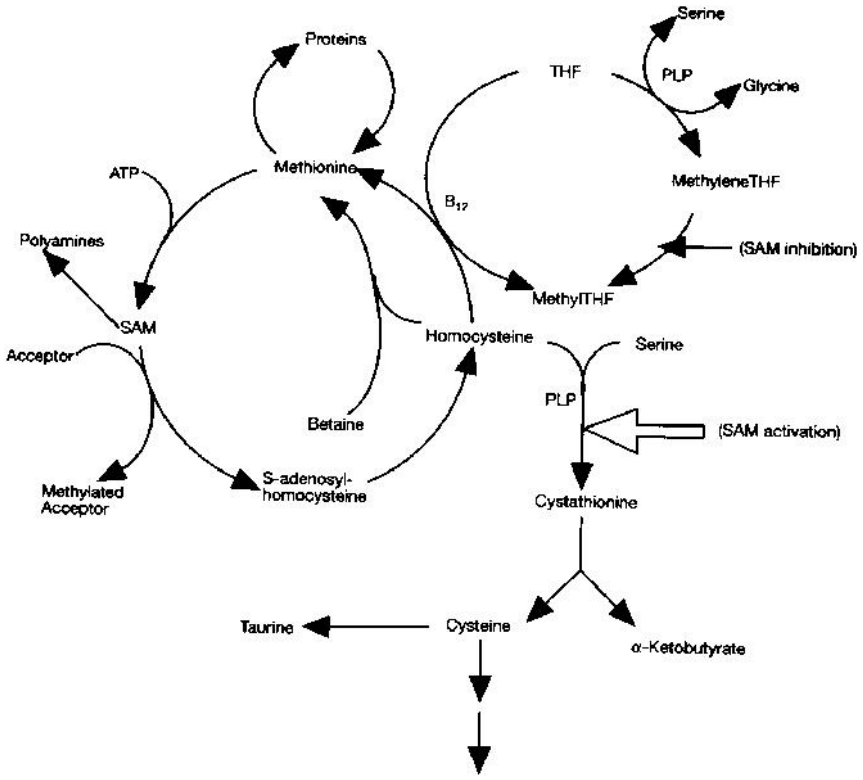


Figure-3 HOMOCYSTEINE METABOLIC PATHWAYS

HYPERHOMOCYSTEINEMIA IN HUMANS:

CAUSES OF HYPERHOMOCYSTEINEMIA[59]
A) ENZYME DEFECTS: 1) Cystathionine synthase (Homocystinuria-1) 2) Homocysteine methyl transferase(Homocystinuria-2) 3) Methyl THFA reductase deficiency(Homocystinuria-3) 4) Cystathionase deficiency (cystathioninuria)
B) ACQUIRED CAUSES 1) Vitamin B12, Folic acid and pyridoxine deficiency. 2) Hypothyroidism, chronic renal failure. 3) Drugs: antifolates, estrogen antagonists.

HOMOCYSTEINE IN NORMAL PREGNANCY:

The normal serum homocysteine level in non pregnant adult female range between 5 and 15 μmol/L. Approximately 75 to 85 percent is protein-bound and 15 to 25 percent is in acid-soluble free forms [60]. Levels of homocysteine are generally lower in pregnancy because of decrease in albumin level, hemodilution or increased demand of methionine both by mother and fetus. Of this, the decrease in serum albumin level correlates well with the serum homocysteine level.

During normal pregnancy the homocysteine levels are significantly lower in all trimesters compared with non pregnant control values. Walker et al[61]. in a study of normal pregnant women and non pregnant women, measured serum homocysteine level by chromatography and found that the lowest level of homocysteine was found during the second trimester and homocysteine raises in the third trimester. However this raise is still well below the normal non pregnant level.[61,62]

The normal level of homocysteine level during normal pregnancy is:

- First trimester- 3.9-7.3μmol/L
- Second trimester-3.5-5.3 μmol/L
- Third trimester- 3.3-7.5 μmol/L [61,62]

HOMOCYSTEINE IN PREECLAMPSIA AND OTHER PREGNANCY RELATED DISORDERS:

Numerous studies have found an association between homocysteine and various pregnancy related disorders. Klai et al[63] assessed the genetic makeup of patients with proven placental vasculopathies and found an association with MTHFRA1298C polymorphism which leads to hyperhomocysteinemia. Elevated homocysteine levels are associated with development of vasculopathy of the placenta. Many hypothesis have been proposed for the pathogenesis of homocysteine induced vasculopathy and endothelial dysfunction.

MECHANISMS BY WHICH HOMOCYSTEINE MAY INDUCE VASCULAR INJURY AND ENDOTHELIAL DYSFUNCTION:

- Homocysteine promotes leukocyte recruitment by upregulating monocyte chemoattractant protein-1 and interleukin-8 expression and secretion.[64]

- The thiolactone metabolite of homocysteine can combine with LDL-cholesterol to produce aggregates that are taken up by vascular macrophages in the arterial intima; these foam cells may then release the lipid into atherosclerotic plaques[65].

- This thiolactone also induces apoptosis in cultured trophoblasts thereby leading to placental dysfunction[65]

- Homocysteine increases smooth muscle cell proliferation and enhances collagen production[66] thereby decreasing uteroplacental flow.

- Prothrombotic effects of homocysteine, include attenuation of endothelial cell tissue plasminogen activator binding sites, activation of factor VIIa and V, inhibition of protein C and heparin sulfate, increased fibrinopeptide A and prothrombin fragments 1 and 2, increased blood viscosity, and decreased endothelial antithrombotic activity due to changes in thrombomodulin function[67-72] - promoting spiral artery thrombosis.

- Oxidative stress by free radicals formed during the oxidation of reduced homocysteine may directly injure endothelial cells[73,74] leading to endothelial cell activation.

- Marked platelet accumulation may be secondary to direct proaggregatory effects of homocysteine or to an impairment in endothelium-mediated platelet inhibition[75,76].

- Prolonged exposure of endothelial cells to homocysteine reduces the activity of dimethylarginine dimethylaminohydrolase, the enzyme that degrades asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase; this impairs the production of nitric oxide[75,77]. This may contribute to impaired endothelium-dependent vasodilation of both conduit and resistance vessels[78,79,80] in the placenta.

- Homocysteine also decreases the invasion in cultured trophoblast by decreasing matrix metalloproteinase-2,-9 expression which are essential for invasion.[81]

HYPERHOMOCYSTEINEMIA IN PREECLAMPSIA

REVIEW OF CLINICAL STUDIES:

Numerous studies have proved the association of hyperhomocysteinemia in preeclampsia.[82-89]. Similarly the level of homocysteine correlated with the severity of preeclampsia[86].

Md.Mazammel hoque et al[82] compared the homocysteine levels in healthy pregnant, preeclamptic and eclamptic pregnant women and found that homocysteine levels are significantly raised in both preeclampsia and eclampsia, but in eclampsia the severity of elevation is more compared to

that in preeclampsia. Mahal M et al[83] compared serum homocysteine and HDL levels in preeclamptic/ eclamptic mother and found that both HDL and homocysteine are increased in women with preeclampsia/eclampsia.

Singh urmila et al[84] studied 90 women and found that homocysteine levels were significantly different between non preeclamptic and preeclamptic mothers . In that study, homocysteine levels correlated directly with the degree of hypertension. And he proved that maternal homocysteine levels in normal pregnant women were significanty lower than non pregnant levels. Similarly, Hasanzadeg et al [85] and Stolkova et al[86] showed that serum homocysteine levels were significantly raised in mothers with preeclampsia.

Ingec et al[89] compared the homocysteine levels among mild and severe preeclampsia with eclampisa and concluded that homocysteine levels are significantly elevated in severe preeclampsia and eclampsia, with significant difference between the mild and severe preeclamptic group. However in that study there was no difference between normal pregnant women and women with mild preeclampsia. Stolkova et al[86] did a study on the distribution of homocysteine levels in preeclamptic women and found that there is an association between serum homocysteine levels and the severity of preeclampsia.

Makedos and papanicollou et al[87] compared maternal homocysteine, folic acid and B12 levels among women with preeclampsia and found that hyperhomocysteinemia is associated with poor maternal and fetal outcome and found that hyperhomocysteinemia occurred in preeclampsia independent of maternal folate and B12 levels.

The Hordaland study[97] showed that women with Homocysteine levels more than 15µ/L are more likely to suffer from pregnancy related complications and are also associated with poor maternal and fetal outcome.

Baksu et al [88] studied the homocysteine levels in pregnancies complicated with severe and non severe preeclampsia and found that at a homocysteine level of above 15µmol/L the maternal and fetal morbidities increased and also proved that homocysteine levels differed significantly among the severe and non severe group.

HYPERHOMOCYSTEINEMIA IN OTHER PREGNANCY RELATED CONDITIONS:

- Hyperhomocysteinemia is found to have some association in neural tube defects.[90]
- Hyperhomocysteinemia in amniotic fluid is associated with small for gestational babies.[91]

- Hyperhomocysteinemia is an independent marker for low birth weight.[92]
- Hyperhomocysteinemia together with uterine artery Doppler could predict IUGR[93] and hyperhomocysteinemia in preeclampsia is associated with IUGR.[87] The Hordaland homocysteine study[97] showed that there is an association between hyperhomocysteinemia and IUGR independent of preeclampsia.
- Hyperhomocysteinemia in preeclampsia is inversely related to insulin sensitivity.[94]
- Hyperhomocysteinemia is a independent risk factor for the development of placental abruption and infarction.[95,97]
- Hyperhomocysteinemia is one of the causes for recurrent pregnancy losses in the first trimester.

MATERIALS AND METHODS

SETTING

This study was conducted in the Department of Obstetrics And Gynaecology at RSRM-Lying in Hospital, Stanley Medical College and Hospital in collaboration with the department of Biochemistry and Clinical pathology.

ETHICAL APPROVAL

Obtained

STUDY DURATION

This study was conducted over a period of 1 year from September 2010 to September 2011.

STUDY POPULATION

Pregnant women attending the RSRM hospital were screened and those who were eligible according to the inclusion criteria and exclusion criteria, were included in the study after obtaining proper detailed consent from the patients.

TYPE OF STUDY

It was a cross sectional case control study.

INCLUSION CRITERIA

- Pregnant women in 28-40weeks who were under regular check up and who are under regular iron and folic acid prophylactic therapy were included in the study.

EXCLUSION CRITERIA

- Chronic hypertension
- Chronic kidney disease/ liver disease
- Diabetes mellitus or Gestational diabetes
- Under anti folate drugs (anti-epileptics)
- Multiple pregnancies and hypothyroid women.
- h/o thromboembolism like deep vein thrombosis etc..
- h/o recurrent miscarriage
- h/o neural tube defects
- presence of megaloblastic or dimorphic anemia in the peripheral smear study.
- Patients who are not under any iron supplements.
- Patients who are not willing to get included in the study.

METHODS

120 patients who came to RSRM are included in the study after obtaining their consent and fulfillment of the inclusion and exclusion criteria. All the patients underwent a detailed history taking and examination as mentioned in the proforma. Then these patients were categorized into three groups as normal pregnancy(group 1) n=40, women with mild preeclampsia(group2)n=40 and women with severe preeclampsia(group3) n=40 according to the American College of Obstetric and Gynaecology guidelines.

GROUP		CRITERIA AS
GROUP 1	Normal pregnant women with singleton pregnancy (28-40 weeks)	BP<140/90
GROUP 2	Women with mild preeclampsia(28-40 weeks)	BP >140/90 BP<160/110 with 1+ proteinuria
GROUP 3	Women with severe preeclampsia (28-40 weeks)	BP ≥ 160/110 with 2+/3+ proteinuria or imminent signs.

Study subjects of each group were apparently matched with respect to gestational age. All these patients were subjected to routine blood investigations like complete hemogram,urine routine, renal function test, liver function test, serum fibrinogen, uric acid, CTG, ultrasonogram and fundus examination. Urinary 24 hrs protein excretion was done for all patients included in the study. Urine cultue were done for the appropriate patients. Peripheral smear study was done for all patients to exclude megaloblastic anemia and dimorphic anemia. Serum folate and vitaminB12 level could not be estimated because of limited resources. Hence the presence of megaloblastic or dimorphic anemia are taken as indirect indictors of folate or B12 deficiency and these patients were ruled out of the study. Once vitamin deficiencies are excluded serum homocysteine level was estimated.

For the measurement of serum homocysteine, 5 mL of blood was drawn from the antecubital vein after overnight fasting and the specimen was transported immediately to lab and centrifugation was done at 3000rpm for 5-7 mts and the clear serum is transferred to a plastic vial and stored in refrigerator until analysis. Serum homocysteine was measured by fluorescence polarization immunoassay (FPIA) run on Abbott's AxSYM machine using Abbott's kit.

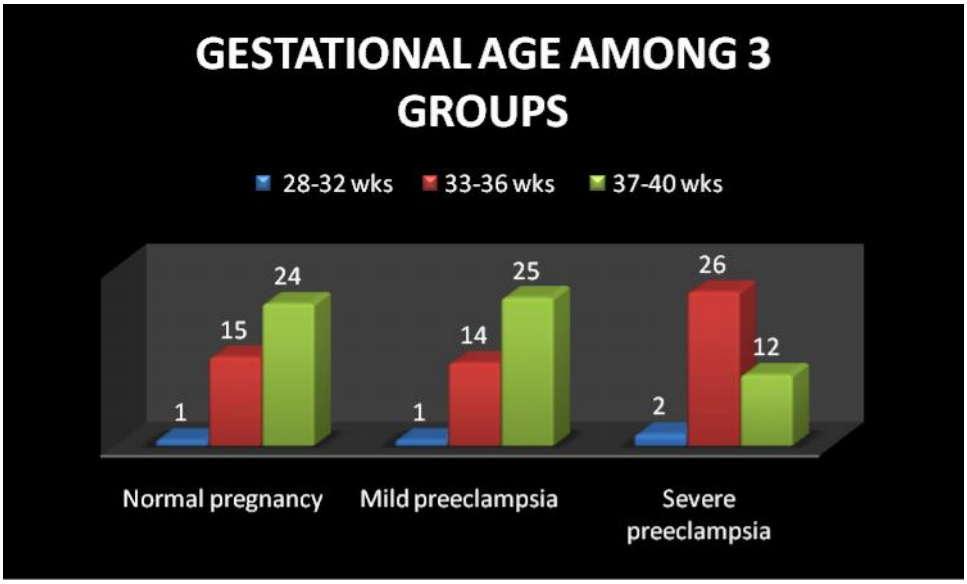
All these patients were followed up till delivery. Maternal morbities like acute kidney injury, pulmonary edema, HELLP syndrome, DIC, abruption, atonic PPH, etc. are watched for and recorded appropriately. Similarly the birth weight, the presence of IUGR, IUD, neonatal mortality were also noted. Also the one minute APGAR score, the duration of NICU stay were noted. The NICU stay was divided into three groups as babies with no NICU stay, stay less than 3 days and stay for more than 3 days. Similarly the maturity of the foetus was also noted. If preterm whether it was spontaneous or iatrogenic was noted. All these values are entered in the master chart for comparison and analysis. A standard proforma was used to record all the variable for every individual patient as given in the annexure-A.

STATISTICAL ANALYSIS:

Statistical analysis was performed with SPSS software (version 15.00 for windows). To find out the statistical significance, one way ANOVA (analysis of variance) test and student ‘t’ test were done. For the purpose of this study 95% confidence interval has been chosen and ‘p’ value <0.05 has been taken as significant.

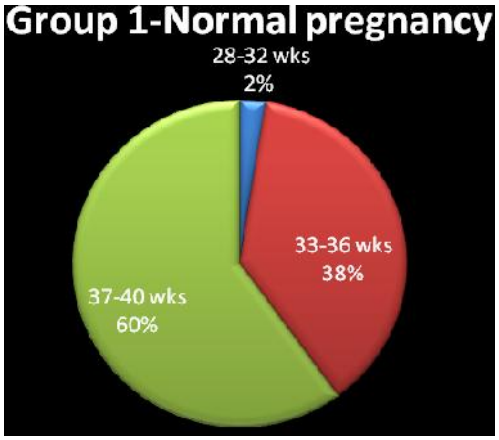
OBSERVATION AND RESULTS

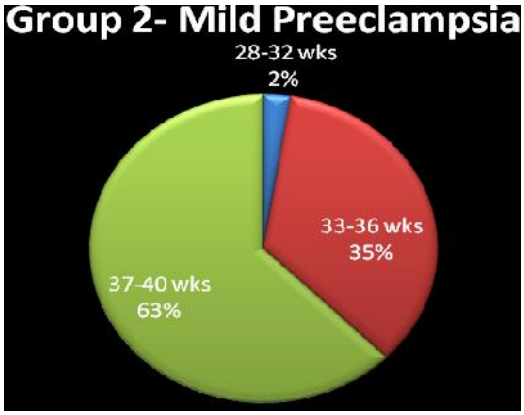
MATERNAL GESTATIONAL AGE DISTRIBUTION AMONG THE GROUPS

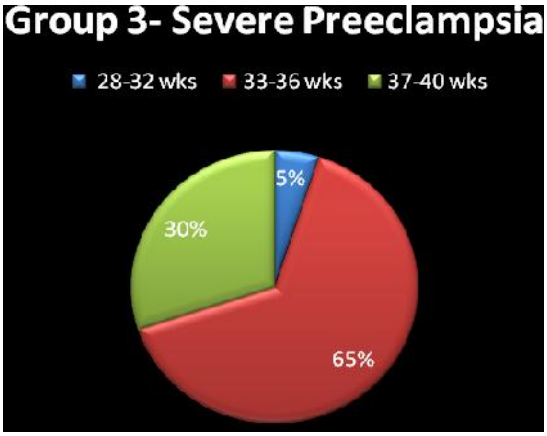


Gestational age	Group 1	Group 2	Group 3
28-32 wks	1	1	2
33-36 wks	15	14	26
37-40 wks	24	25	12
MEAN GESTATIONAL AGE	36.58	36.45	35.9

MATERNAL GESTATIONAL AGE AMONG THE 3 GROUPS







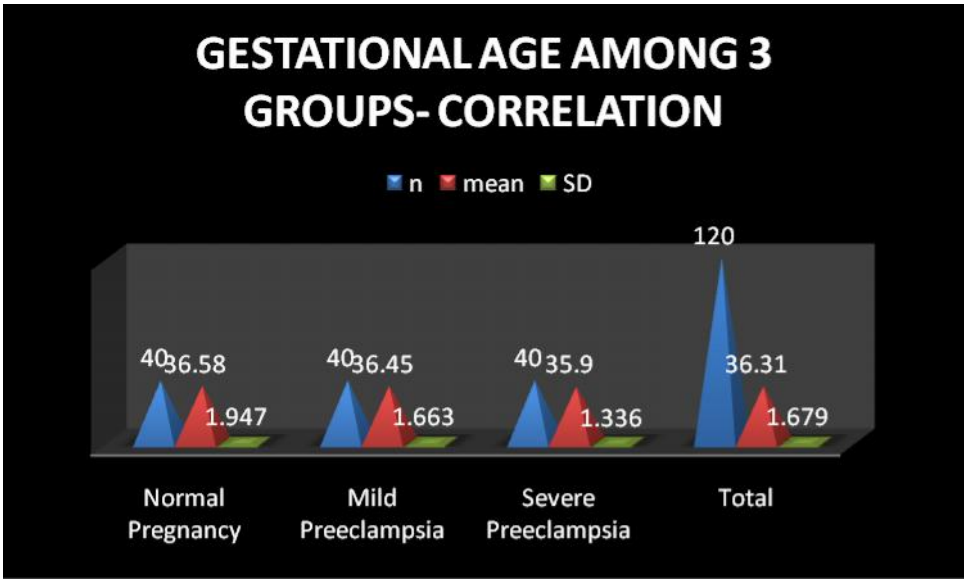
COMPARISON OF MATERNAL GESTATIONAL AGE BETWEEN GROUP 1,2 AND 3 – ANOVA TEST

GESTATIONAL AGE	GROUPS	N	MEAN	S.D
	Normal Pregnancy (GROUP 1)	40	36.58	1.947
	Mild Preeclampsia (GROUP 2)	40	36.45	1.663
	Severe Preeclampsia (GROUP 3)	40	35.90	1.336
	Total	120	36.31	1.679

		Sum of Squares	df	Mean Square	F	Sig.	p value
GA	Between Groups	10.317	2	5.158	1.855	.161	> 0.05
	Within Groups	325.275	117	2.780			
	Total	335.592	119				

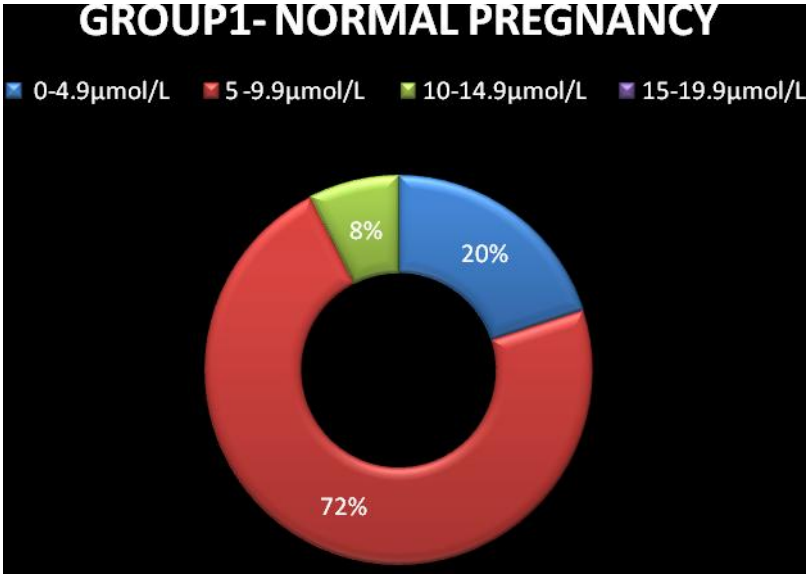
POSTHOC TEST MULTIPLE COMPARISONS

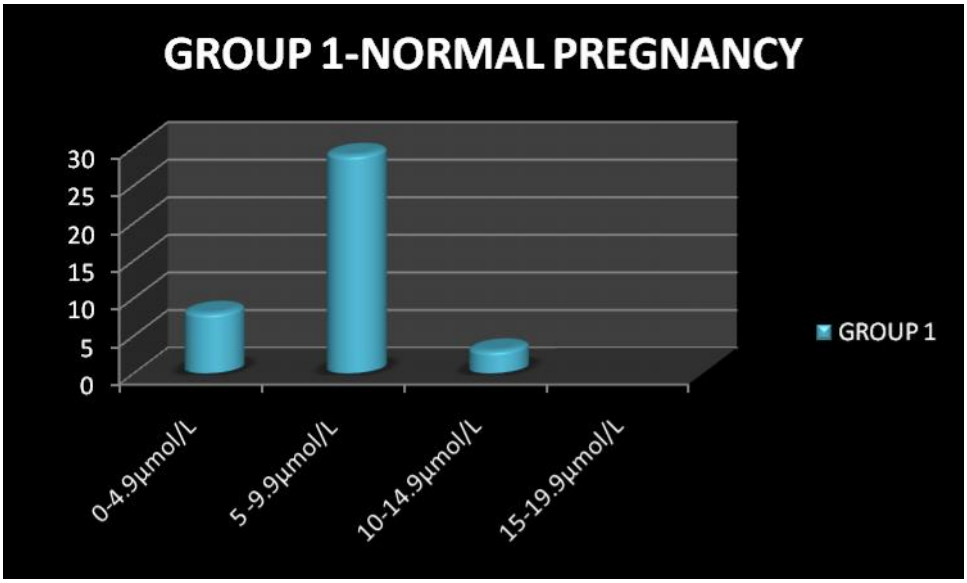
Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	p VALUE
GESTATIONAL AGE	Normal Pregnancy	Mild Preeclampsia	.13	.373	.940	> 0.05
		Severe Preeclampsia	.68	.373	.171	> 0.05
	Mild Preeclampsia	Normal Pregnancy	-.13	.373	.940	> 0.05
		Severe Preeclampsia	.55	.373	.307	> 0.05
	Severe Preeclampsia	Normal Pregnancy	-.68	.373	.171	> 0.05
		Mild Preeclampsia	-.55	.373	.307	> 0.05



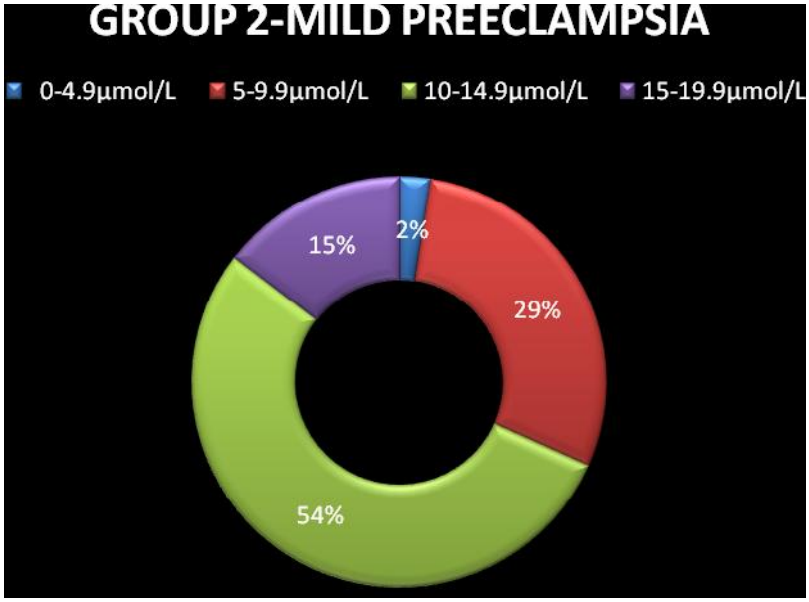
In our study there was no statistically significant difference among the three groups with respect to the maternal gestational age.

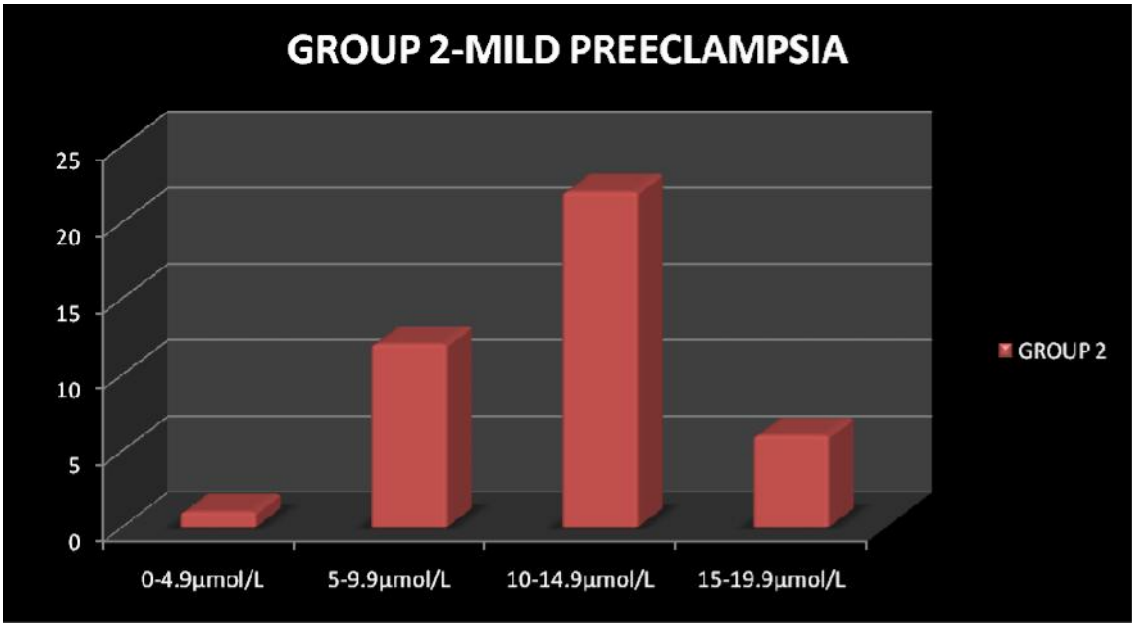
HOMOCYSTEINE LEVEL AMONG GROUP 1-NORMAL PREGNANT WOMEN



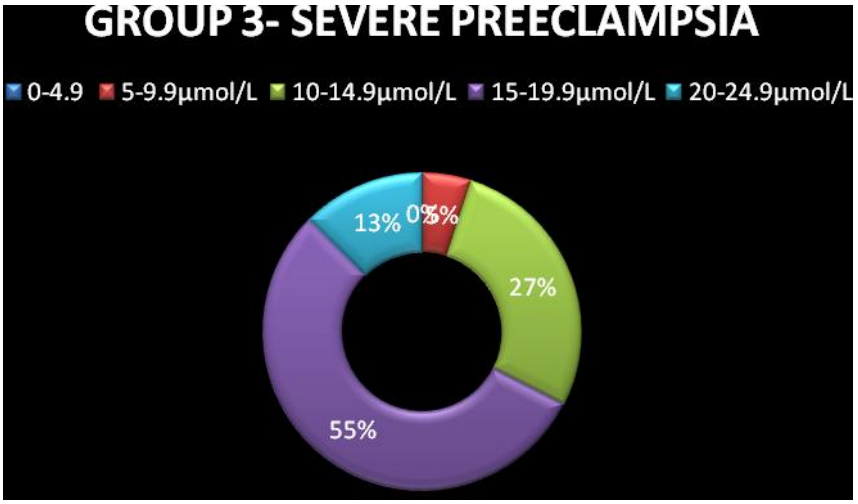


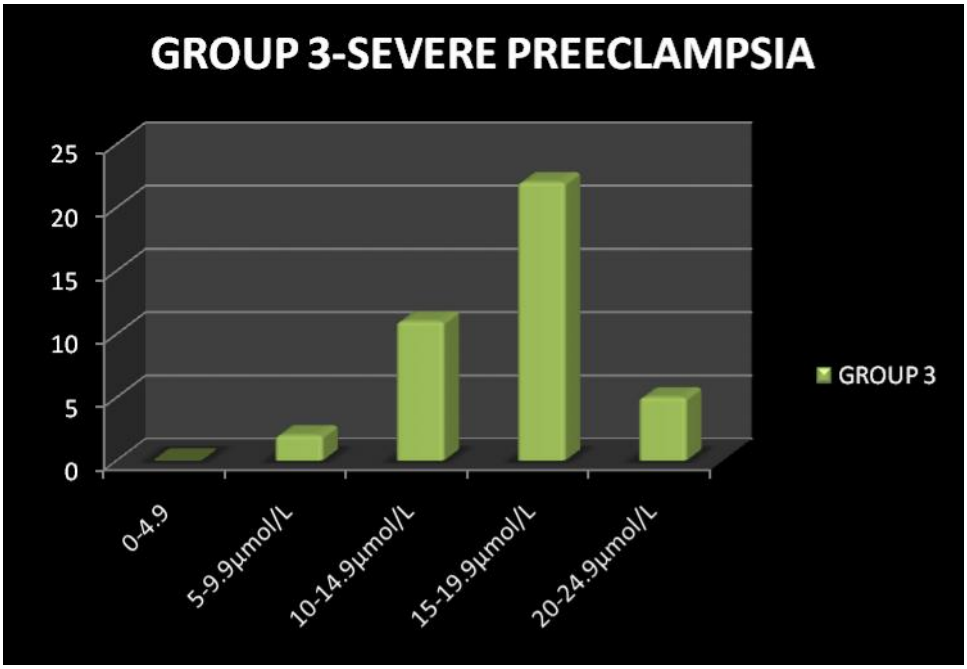
HOMOCYSTEINE LEVEL AMONG GROUP 2-MILD PREECLAMPSIA



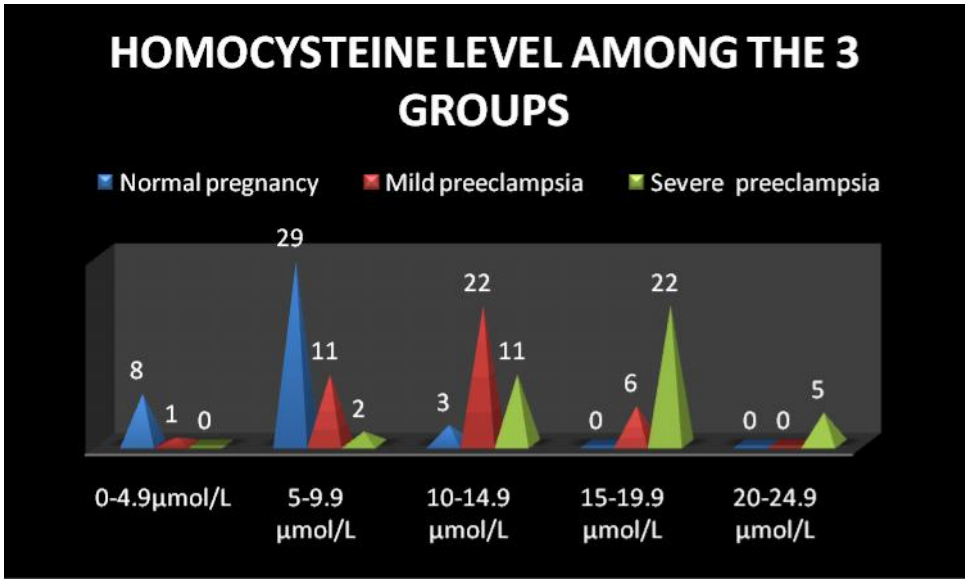


HOMOCYSTEINE LEVEL AMONG GROUP 3-SEVERE PREECLAMPSIA





CORRELATION OF MATERNAL HOMOCYTEINE AMONG THE THREE GROUPS



		N	Mean	Std. Deviation
Homocysteine	Normal Pregnancy	40	6.650	1.5785
	Mild Preeclampsia	40	11.583	2.3770
	Severe Preeclampsia	40	16.670	3.3467
	Total	120	11.634	4.8181

CORRELATION OF MATERNAL HOMOCYTEINE AMONG THE THREE GROUPS

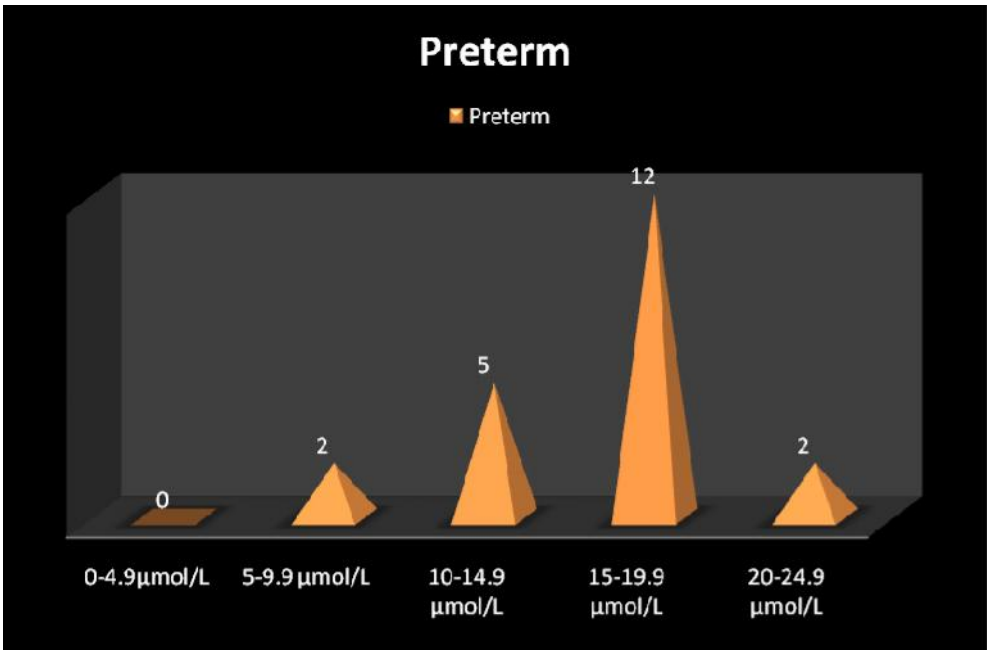
		Sum of Squares	df	Mean Square	F	Sig.	p value
Homocysteine	Between Groups	2008.168	2	1004.084	155.736	.000	< 0.001
	Within Groups	754.342	117	6.447			
	Total	2762.510	119				

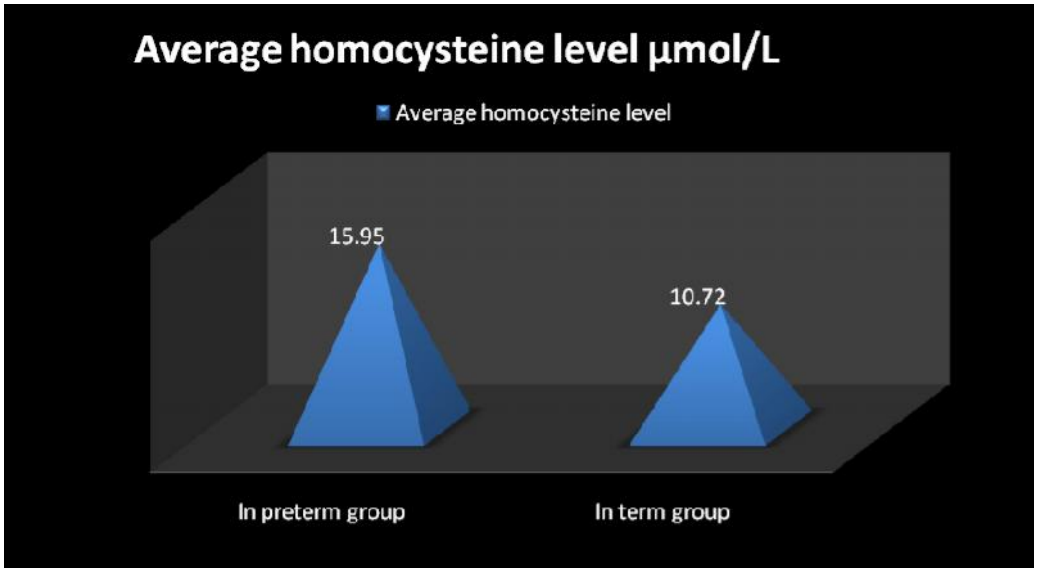
Post Hoc Tests Multiple Comparisons

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	p value
Homocysteine	Normal Pregnancy	Mild Preeclampsia	-4.932	.5678	.000	<0.001
		Severe Preeclampsia	-10.020	.5678	.000	<0.001
	Mild Preeclampsia	Normal Pregnancy	4.932	.5678	.000	<0.001
		Severe Preeclampsia	-5.087	.5678	.000	<0.001
	Severe Preeclampsia	Normal Pregnancy	10.020	.5678	.000	<0.001
		Mild Preeclampsia	5.087	.5678	.000	<0.001

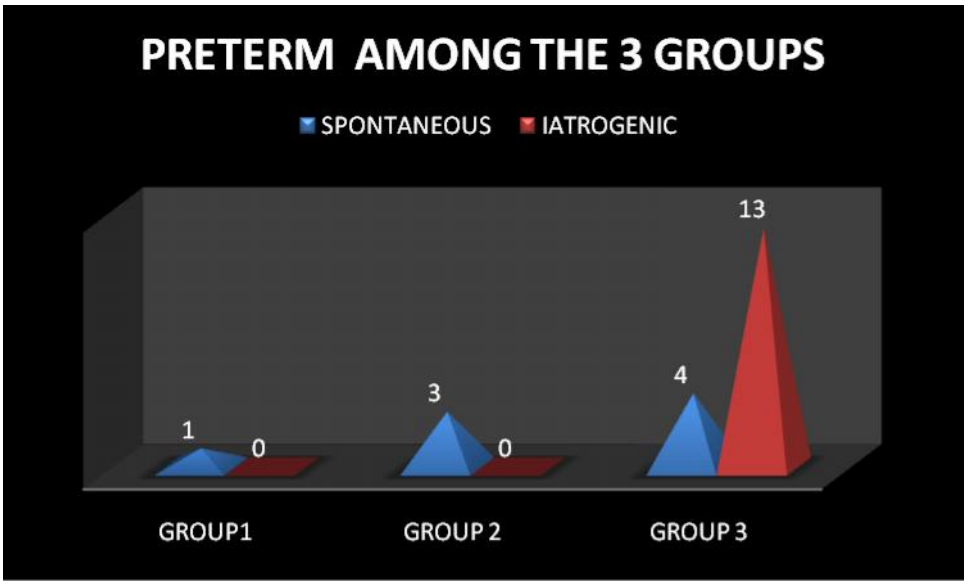
In our study there was a significant difference between the homocysteine levels among all the three groups.

CORRELATION OF MATERNAL HOMOCYSTEINE LEVEL WITH FETAL MATURITY





PRETERM DISTRIBUTION AMONG THE 3 GROUPS

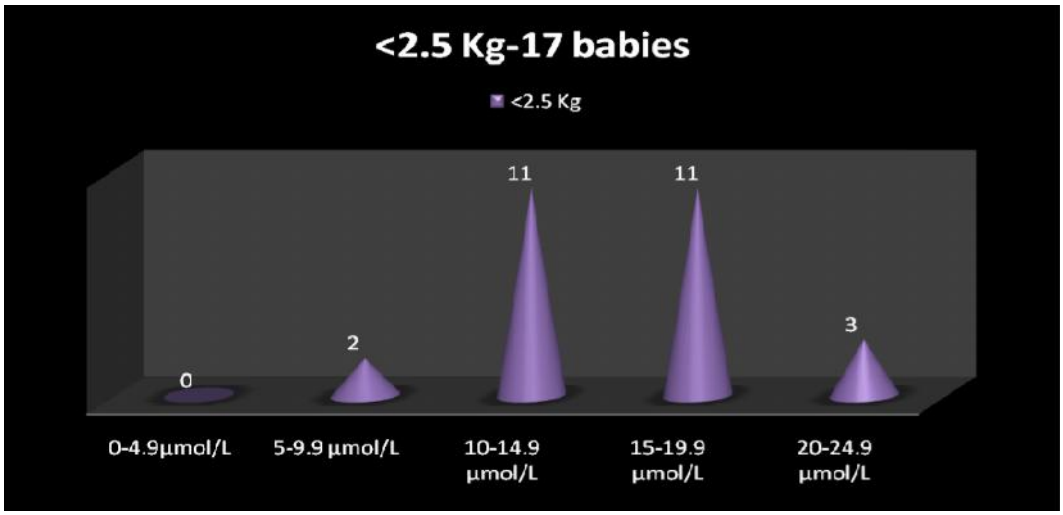


CORRELATION OF MATERNAL HOMOCYSTEINE LEVEL WITH FETAL MATURITY

	n	**Average homocysteine level**	**STD Deviation**	t	df	Sig. (2-tailed)	p value
In preterm group	21	15.95	4.08	4.948	118	.000	< 0.001
In term group	99	10.72	4.47	5.249	31.045	.000	< 0.001

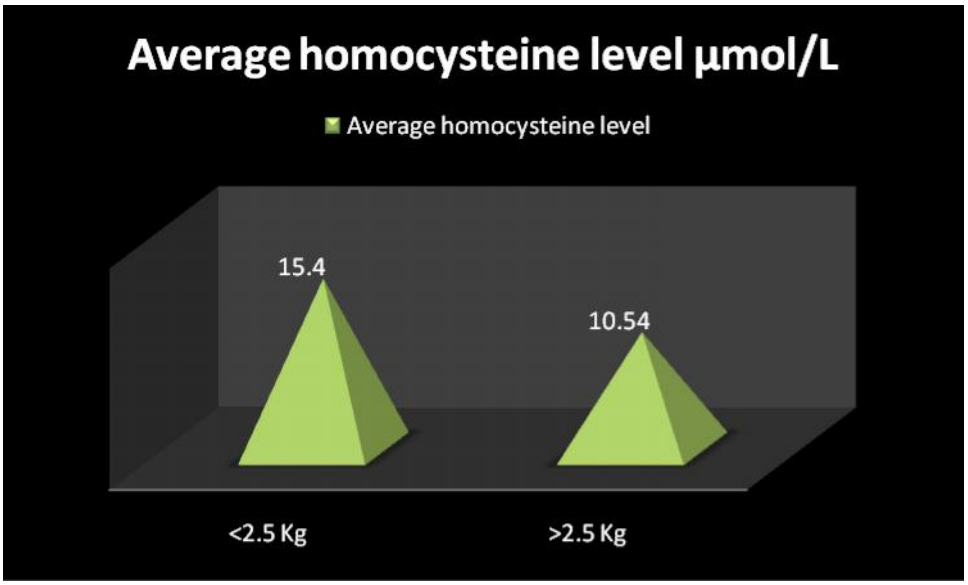
In our study, there is an association between maternal homocysteine level and fetal maturity.Most of the prematurity in the severe preeclamptic group (76%) was iatrogenic.

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE LEVEL AND BIRTH WEIGHT



homocysteine level	0-4.9µmol/L	5-9.9 µmol/L	10-14.9 µmol/L	15-19.9 µmol/L	20-24.9 µmol/L
<2.5 Kg	0	2	11	11	3

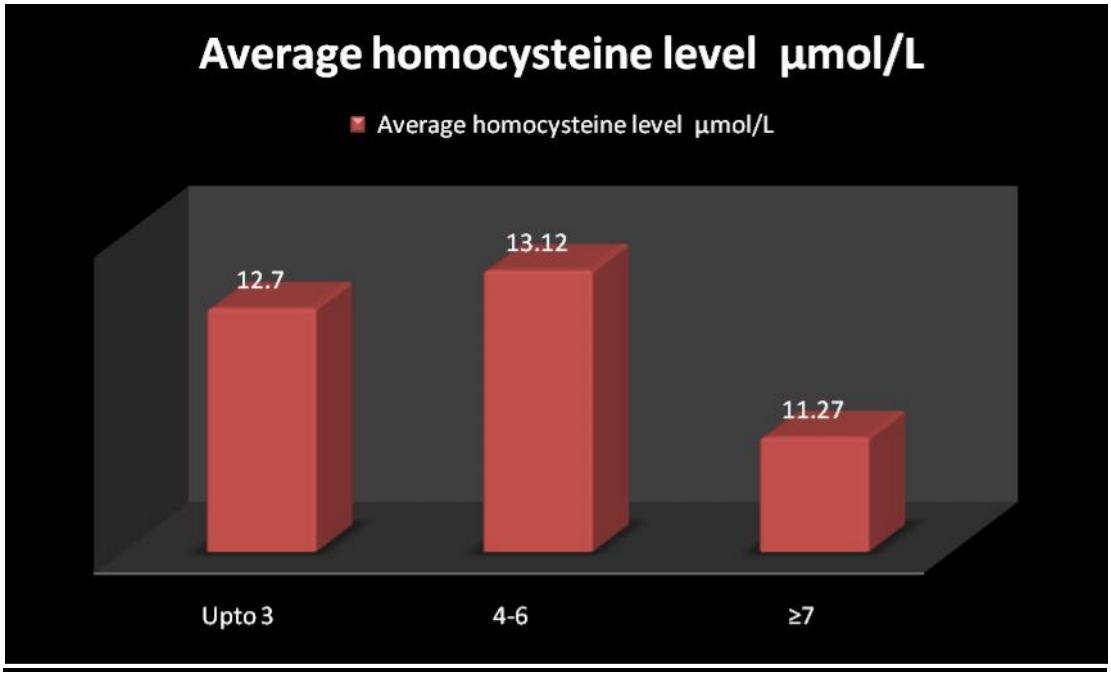
CORRELATION BETWEEN MATERNAL HOMOCYSTEINE LEVEL AND BIRTH WEIGHT



	n	**Average homocysteine level**	**STD Deviation**	t	df	Sig. (2-tailed)	p value
<2.5 Kg	27	15.4	4.19	5.070	118	.000	< 0.001
>2.5 Kg	93	10.54	4.44	5.228	44.291	.000	

In our study, there is an association between maternal homocysteine level and the neonatal birth weight.

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE LEVEL AND 1 MINUTE APGAR SCORE



APGAR SCORE	Average homocysteine level µmol/L	n
Upto 3	12.7	4
4-6	13.12	13
7-10	11.27	101 (IUD=2)

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE LEVEL AND 1 MINUTE APGAR SCORE

ANOVA

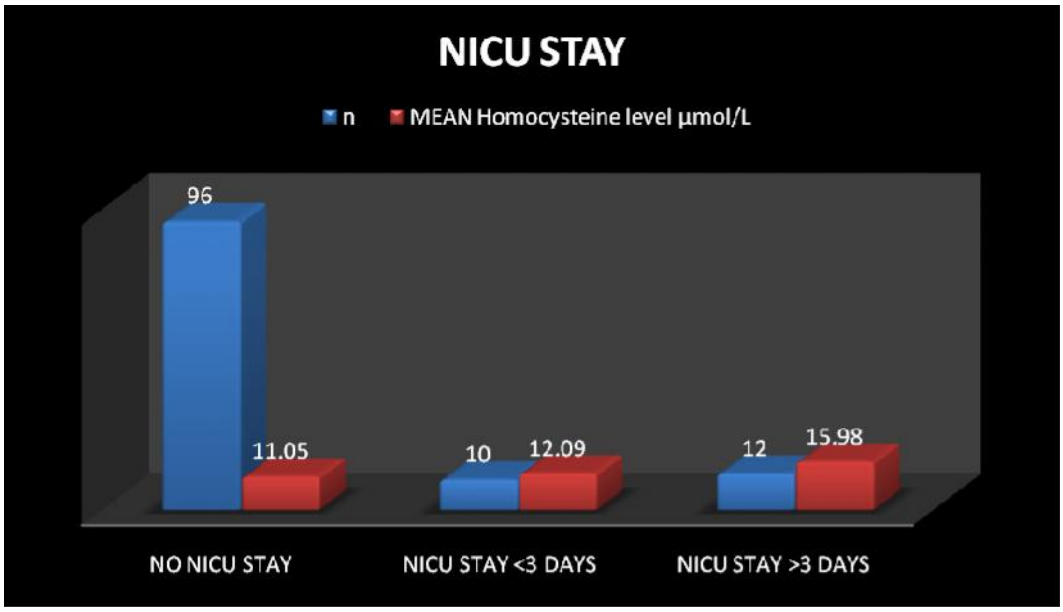
	Sum of Squares	df	Mean Square	F	Sig.	p value
Between Groups	45.157	2	22.578	.995	.373	
Within Groups	2608.822	115	22.685			>0.05
Total	2653.979	117				

POST HOC TESTS MULTIPLE COMPARISONS

(I) Apgar	(J) Apgar	Mean Difference (I-J)	Std. Error	Sig.	p value
≤ 3	4-6	-.415	2.7233	.987	> 0.05
	≥ 7	1.434	2.4282	.826	> 0.05
4-6	≤ 3	.415	2.7233	.987	> 0.05
	≥ 7	1.849	1.4034	.388	> 0.05
≥ 7	≤ 3	-1.434	2.4282	.826	> 0.05
	4-6	-1.849	1.4034	.388	> 0.05

In our study, there was no association between maternal homocysteine level and 1 minute APGAR score.

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE LEVEL AND DURATION OF NICU STAY



	NO NICU STAY	NICU STAY <3 DAYS	NICU STAY >3 DAYS
No of babies n	96	10	12
MEAN Homocysteine level µmol/L	11.05	12.09	15.98

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE LEVEL AND DURATION OF NICU STAY

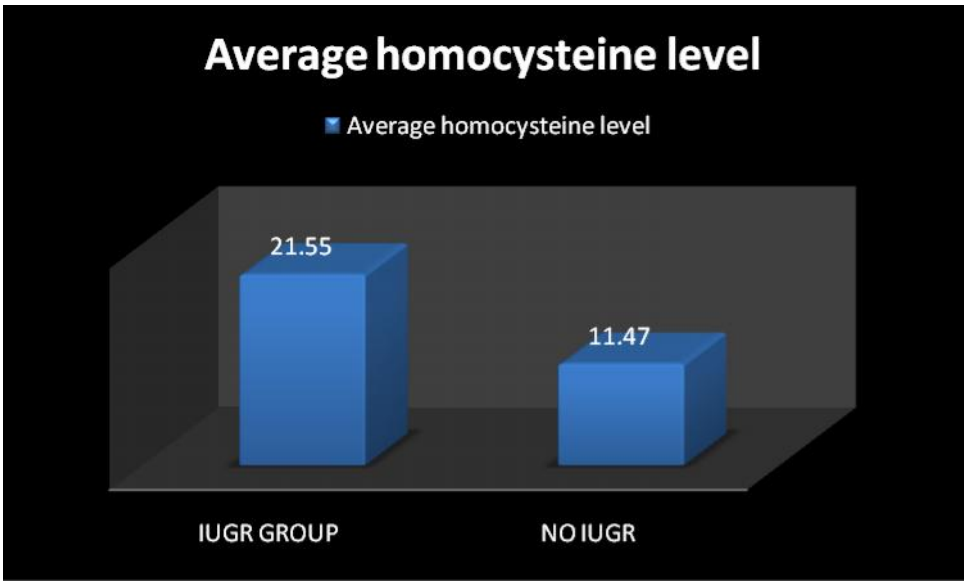
	n	**Average homocysteine level**	**STD Deviation**	t	df	Sig. (2-tailed)	p value
No nicu	96	10.9	4.65	-.772	104	.442	> 0.05
<3 days nicu	10	12.09	4.43	-.804	11.171	.438	

	n	**Average homocysteine level**	**STD Deviation**	t	df	Sig. (2-tailed)	p value
No nicu	96	10.9	4.65	-3.645	106	.000	< 0.001
>3 days nicu	12	15.98	3.59	-4.450	15.996	.000	

	n	**Average homocysteine level**	**STD Deviation**	t	df	Sig. (2-tailed)	p value
NICU< 3 DAYS	10	12.09	4.43	-2.276	20	.034	< 0.05
Nicu> 3 days	12	15.98	3.59	-2.232	17.327	.039	

In our study, there was no association between homocysteine level and NICU stay for less than 3 days. However there is an association between maternal homocysteine level and babies requiring NICU stay for more than 3 days.

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE AND IUGR

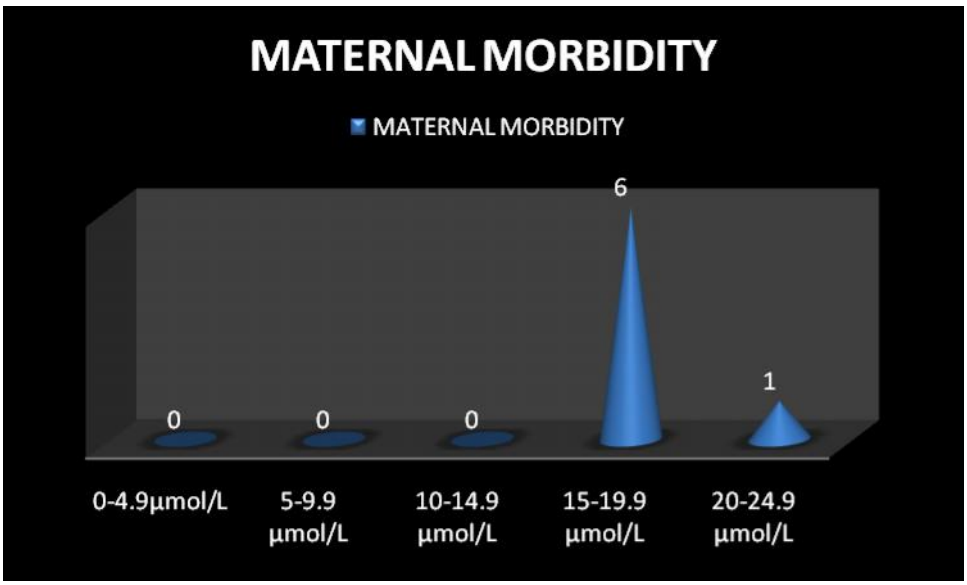


	n	Average homocysteine level	STD Deviation	t	df	Sig. (2-tailed)	p value
IUGR	2	21.55	3.46	3.035	118	.003	< 0.01
OTHERS	118	11.47	4.67	4.054	1.062	.143	

In our study , there is an association between maternal homocysteine level and fetal IUGR.

CORRELATION OF HOMOCYSTEINE WITH MATERNAL

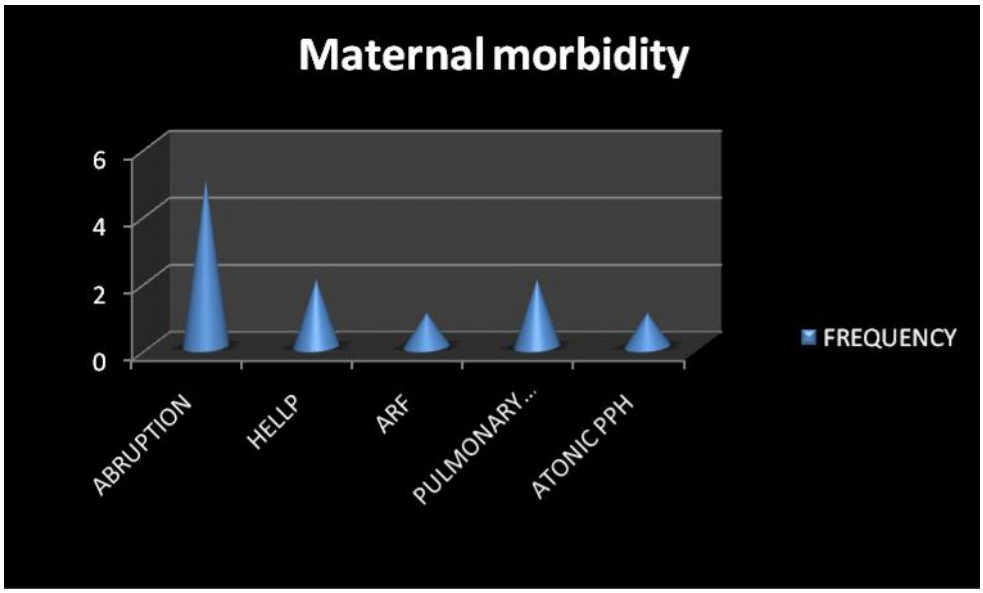
MORBIDITY



Homocysteine level	0-4.9µmol/L	5-9.9 µmol/L	10-14.9 µmol/L	15-19.9 µmol/L	20-24.9 µmol/L
MORBIDITY	0	0	0	6	1

In our study all the maternal morbidities occurred in mothers with homocysteine level of more than 15µ/L.

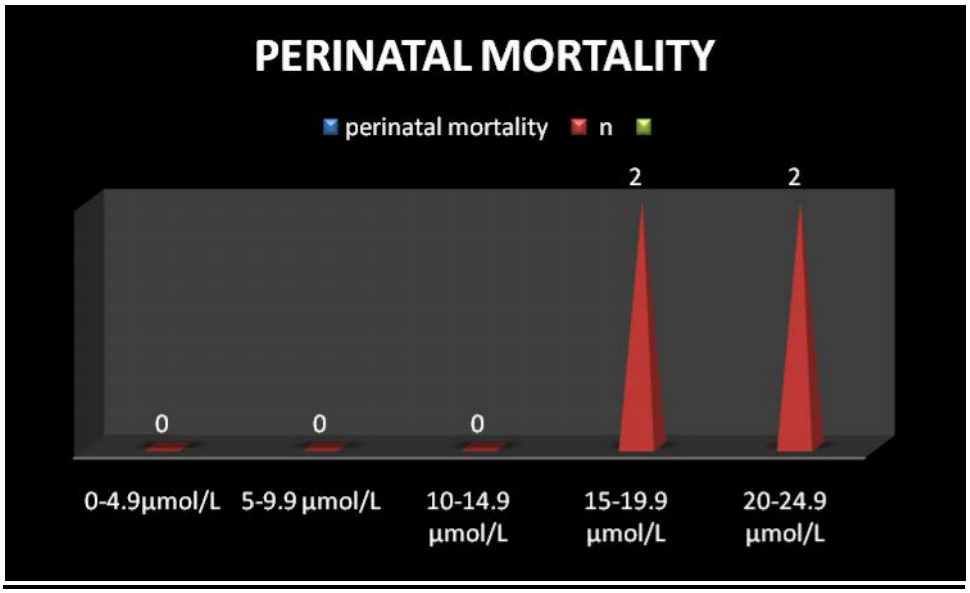
CORRELATION OF HOMOCYSTEINE WITH MATERNAL MORBIDITIY



	n	**Average homocysteine level**	**STD Deviation**	t	df	Sig. (2-tailed)	p value
Maternal morbidity	7	18.38	1.83	4.062	118	.000	< 0.001
Normal	113	11.21	4.63	8.774	11.619	.000	

In our study, there is an association between maternal homocysteine level and various maternal morbidities in preeclamptic mothers.

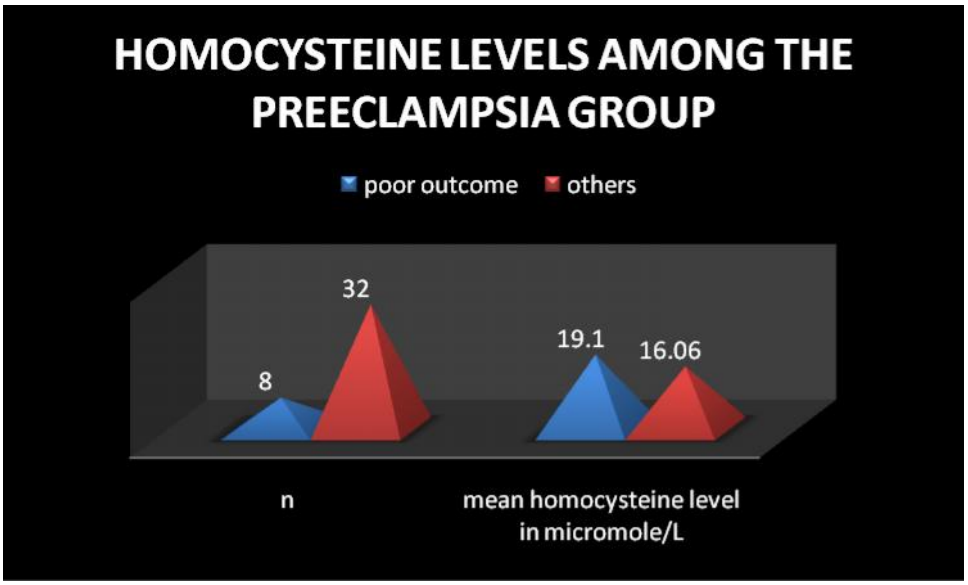
CORRELATION OF MATERNAL HOMOCYSTEINE LEVEL WITH PERINATAL MORTALITY



	n	Average homocysteine level	STD Deviation	t	df	Sig. (2-tailed)	p value
Perinatal mortality	4	18.85	2.27	3.159	118	.002	< 0.01
Normal	116	11.38	4.69	6.128	3.942	.004	

In our study, there is an association between maternal homocysteine level and fetal perinatal mortality.

CORRELATIION OF HOMOCYSTEINE LEVEL WITH VERY POOR MATERNAL AND FETAL OUTCOMES IN THE SEVERE PREECLAMPSIA GROUP



GROUP 3	n	MEAN	S.D	variance	p VALUE
Very poor outcome	8	19.6	2.61	6.81	< 0.001
Others	32	16.06	3.26	10.6	

In our study homocysteine levels (in the severe preeclamptic group) differed significantly among mothers with poor maternal and fetal outcome from others.

DISCUSSION

In our study 120 patients were included after fulfilling the inclusion and exclusion criteria with 40 patients in each group- normal pregnany, mild preeclampsia and severe preeclampsia.

GESTATIONAL AGE AMONG THE THREE GROUPS:

The average gestational age for the 3 groups were 36.58 wks for group 1, 36.45 wks for group2 and 35.9 wks for group 3. There was no statistically significant difference in the gestational age of the three groups ($p > 0.05$). In our study 51% of women were in 36-40 weeks of gestation, 45% were between 33-36 weeks of gestation and 4% were between 28-32 weeks of gestation.

HOMOCYSTEINE DISTRIBUTION AMONG THE 3 GROUPS:

TABLE-1

GROUP	MEAN homocysteine level (µmol/L)	RANGE	mean± 2SD
GROUP 1 (Normal pregnancy)	6.65	4.3-10.7	5.07-8.23
GROUP 2 (Mild preeclampsia)	11.58	4.7-15.5	9.2-13.96
GROUP 3 (severe preeclampsia)	16.67	9.5-24.0	13.32-20.02

Table-1 shows the homocysteine level in the different groups. In our study the mean homocysteine level among normal pregnant mothers was 6.65 µmol/L. this is almost same as the homocysteine level as 6.86 µmol/L in the study conducted by mozammel hoque et al[82]. In another study done at Canada the mean homocysteine level at 36-42wks among normal pregnant women was 5.5 µmol/L [61]. In the study conducted by Stolkova et al[86] the mean homocysteine level was 6.24 µmol/L almost similar to our study. In our study the mean homocysteine level in patients with mild preeclampsia was 11.58 ±2.38 µmol/L. In the study done by hasanzadeh et al[85] the homocysteine level among mild preeclampsia was 10.4±2.3 which is close to our study.

In our study the homocysteine level among the severe preeclampsia group was 16.67±3.35 µmol/L. This is close to the study of hasanzadeh et al [85] where the homocysteine level was 13.8±7. In another study by ingec et al[89] the homocysteine level among severe preeclamptic women was 16.7±10.1 µmol/L which is almost similar to our study.

CORRELATION OF HOMOCYSTEINE LEVEL WITH PREECLAMPSIA:

On comparing group 1 and 2, in our study there was a statistically significant difference between the homocysteine level among normal

pregnant women and women with mild preeclampsia (p <0.001) . Similarly between group 1 and group 3 the serum homocysteine level differed significantly (p<0.001) . So, our study proves that serum homocysteine is raised in preeclampsia and there is an association between preeclampsia and hyperhomocysteinemia. Several studies have proven this concept that preeclampsia is associated with hyperhomocysteinemia[82-90]. The large Hordaland homocysteine - a population based study[97] done over a period of 7 years in a population of about 7,053, concluded that hyperhomocysteinemia is a risk factor for preeclampsia.

CORRELATION OF HYPERHOMOCYSTEINEMIA WITH SEVERITY OF PREECLAMPSIA:

Also in our study there was a significant difference in homocysteine level between mothers with mild and severe preeclampsia (p<0.001). Similar findings were also noted in the study conducted by Stolkova et al[86] .where maternal homocysteine level correlated with the severity of preeclampsia. Our study shows that maternal homocysteine level is directly associated with the severity of preeclampsia. In the study by Ingec et al[89] there was a significant difference between mild and severe preeclampsia, however homocysteine levels did not vary significantly between normal pregnant women and women with mild preeclampsia.

Baksu et al [88] in his study showed that levels differed significantly among the severe and non severe group. However another study by Hasanzadeh et al[85] failed to show an association between the severity of preeclampsia and maternal homocysteine level although this study confirmed hyperhomocysteinemia in preeclampsia[85]. These findings points that hyperhomocysteinemia due to its direct vascular endothelial injury and prothrombotic effect on the coagulatory system has a potential role in the pathogenesis of preeclampsia.

CORRELATION OF HOMOCYSTEINE LEVEL WITH FETAL MATURITY:

In our study, 21 out of 120 fetus were preterm. Out of this 21, 17 preterm births (81%) occurred in the severe preeclampsia group , 3 (18%) in the mild preeclamptic group and 1(1%) in the normal pregnancy group. There were no iatrogenic preterm birth in the normal pregnancy and mild preeclamptic group. However 14 out of the 17 preterm births in the severe preeclampsia group (82%) are due to iatrogenic preterm birth primarily done to terminate pregnancy. In our study the incidence of prematurity among the preeclamptic group was 25%. This is almost same as reported by Das et al (1998) as 27%. According to Mudaliar, Menon, toxemias account for 22% of preterm delivery .The maternal homocysteine level was

significantly elevated in the preterm group ($p < 0.001$) when compared to the term group.The Hordaland homocysteine study done at western Norway showed that hyperhomocysteinemia in a population is a risk factor for preterm/prematurity[97] of the fetus.

CORRELATION OF HOMOCYSTEINE LEVEL WITH BIRTH WEIGHT:

In our study 27 new borns were of low birth weight. The average homocysteine level of the mothers of these low birth weight babies was 15.4 μmol/L. This was statistically different from the mean homocysteine level of the rest of the mothers -10.4 μmol/L($p<0.001$). Our study shows that there is a direct association between maternal hyperhomocysteinemia and fetal birth weight. In the study by Baksu et al[88], maternal homocysteine level cut-off value of 15μ/L correlated well with mothers with low birth weight babies. In a study done by grandone et al[91] , it was shown that hyperhomocysteinemia in amniotic fluid was associated with small for gestational age babies and low birth weight independent of preeclampsia. The Hordaland Homocysteine study[97] showed that hyperhomocysteinemia is a risk factor for low birth weight.

CORELATION OF MATERNAL HOMOCYSTEINE LEVEL WITH 1 MINUTE APGAR SCORE:

In our study, we divided the neonates into 3 groups based on their 1 minute APGAR score as group1(APGAR≤ 3), group 2(APGAR 4-6) and group 3 (APGAR ≥ 7). Though to the best of our knowledge we could not find any studies comparing maternal homocysteine level with APGAR score , we tried to find out whether there was any association between the two. However, on comparing we could not find any statistically significant difference between the three groups ($p>0.05$). In our study there is no association between maternal homocysteine levels and APGAR score done at 1 minute.

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE AND DURATION OF NICU STAY

We divided the neonates into 3 groups based on the need for NICU stay. Group 1 (no NICU stay), group 2(NICU stay ≤ 3 days) and group 3 (NICU stay > 3 days) and tried to correlate the hyperhomocysteinemia with these three groups. On analysis, we found that there is no association between homocysteine levels and the need for NICU for ≤ 3 days. However the level of homocysteine was significantly higher in mothers of neonates who required more than 3 days of NICU care. We could not find

any study correlating homocysteine with duration of NICU stay. From our study, maternal hyperhomocysteine can predict a group of mothers who might require prolonged neonatal care for their babies.

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE LEVEL AND FETAL IUGR:

In our study, there was an association between maternal hyperhomocysteinemia and fetal IUGR ($p<0.01$). This is not surprising because of the known effects of homocysteine on placenta in the placental vasculopathy[63]. Also homocysteine decreases the invasion of trophoblasts[81]. However preeclampsia by itself can produce IUGR. The Hordaland Homocysteine study[97] showed that maternal hyperhomocysteinemia due to MTHFR 677C.T polymorphism is associated with fetal growth restriction.

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE LEVEL AND MATERNAL MORBIDITIES:

Studies have shown an association between maternal hyperhomocysteinemia and abruption[95,97]. In our study 7 mothers in the severe preeclampsia group suffered from maternal morbidities like

abruption, acute kidney injury, pulmonary edema, HELLP syndrome and atonic post partum hemorrhage. On comparing the homocysteine level we found that there is significant association between maternal homocysteine level and later morbidities($p<0.001$). All the 7 mothers had a homocysteine level of more than 15µmol/L and all these morbidities occurred only in severe preeclampsia. This is similar to the study of Baksu et al[88] where a cut-off value of 15µmol/L for maternal homocysteine level predicted all maternal morbidities. Larger studies are needed to confirm the level of maternal homocysteine level which can exactly predict the maternal morbidities and mortality. Hyperhomocysteinemia has been accepted as a cause of placental abruption .[97,98] In our study there was no maternal mortality.

CORRELATION BETWEEN MATERNAL HOMOCYSTEINE LEVEL AND PERINATAL MORTALITY:

In our study the number of perinatal deaths was four. 2 were due to intra uterine deaths and 2 were due to neonatal mortality. There was a significant difference between homocysteine level of mothers with perinatal death and without perinatal death ($p<0.01$). The Hordaland Homocysteine study showed that there is an association between hyperhomocysteinemia and still births and adverse pregnancy outcomes.[97]

Similar to the study of Baksu et al[88] , a cut-off value of maternal homocysteine level of 15µmol/L in our study, had a correlation with the perinatal mortality.

CORRELATIION OF HOMOCYSTEINE LEVEL WITH VERY POOR MATERNAL AND FETAL OUTCOMES IN THE SEVERE PREECLAMPSIA GROUP:

In our study, we tried to compare the homocysteine levels among the severe preeclampsia group . We divided the severe preeclampsia group into mothers with very poor maternal and fetal outcomes like complications of preeclampsia, IUD,IUGR and neonatal mortality and mothers without these features. We compared the homocysteine level among these two groups and found that homocysteine level was significantly elevated in mothers with poor maternal and fetal outcomes in the severe preeclampsia group. This suggests that hyperhomocysteinemia per se is associated with poor outcomes independent of preeclampsia. However large studies are needed in pregnancies before attributing hyperhomocystinemia to such an outcome.

SUMMARY OF THE STUDY

120 pregnant women were selected for our study. There were three groups in our study as women with normal pregnancy, women with mild preeclampsia and women with severe preeclampsia. These women were included after they fulfilled the inclusion and exclusion criteria. Serum homocysteine level was estimated in all the women and all of them were followed upto pregnancy and maternal and fetal outcomes were recorded.

The mean age of women in group 1 was 36.58 weeks , 36.45 weeks for group 2 and 35.9 weeks for group 3. There was no statistically significant difference in the age of the three groups.The homocysteine level among the normal pregnant women was 6.65±3.16 μmol/L, 11.58±4.76 μmol/L for the mild preeclmaptic group and 16.67±6.7 μmol/L for women with severe preeclampsia. Women with severe preeclampsia had the highest homocysteine level followed by mild preeclampsia and then normal pregnant women. All these values were statistically significant implying that homocysteine level differed significantly between women with and without preeclampsia and also correlated with the severity of preeclampsia.

With respect to the maternal and fetal outcome, elevated homocysteine levels correlated with all maternal morbidities. All these morbidities

occurred in mothers with homocysteine levels of more than 15 µmol/L . The average homocysteine levels in the morbidity group was 18.38±3.66 µmol/L and the group with no morbidity had a homocysteine level of 11.21± 9.26 µmol/L Elevated maternal homocysteine levels were associated with increased fetal IUGR, fetal preterm birth, low birth weight, prolonged NICU stay of the neo-nate and increased perinatal mortality. All the mothers who had IUD, neonatal mortality and IUGR had a homocysteine level of more than 15 µmol/L. However maternal homocysteine level did not correlate with the 1 minute APGAR score of the newborn and also with newborns who needed NICU stay of less than 3 days. Even among the severe preeclamptic women, hyperhomocysteinemia per se can cause poor maternal and fetal outcomes independent of preeclampsia.

CONCLUSIONS FROM THE STUDY

- Preeclampsia is associated with Hyper homocysteinemia.
- In preeclampsia, homocysteine levels are directly related to the severity of preeclampsia.
- A maternal homocysteine level of more than 15μmol/L can be used to expect poor maternal and fetal outcome until further larger studies predict the exact value.
- Maternal hyperhomocysteinemia is associated with prematurity, low birth weight and increased perinatal mortality.
- Maternal hyperhomocysteinemia can predict a group of mothers whose babies might require special prolonged neonatal care
- Hyperhomocysteinemia increases maternal morbidities especially placental abruption.
- There is no association between maternal homocysteine level and neonatal APGAR score done at 1 minute.
- Similarly maternal hyperhomocysteinemia cannot predict babies who need ≤ 3 days of NICU care from babies whom may not need NICU care.

- Since nutritional deficiencies like B12, folate and B6 are known to produce hyperhomocysteinemia, better nutritional care to the at risk mothers should be done till further studies confirm the role of hyperhomocysteinemia in preeclampsia.

- Though hyperhomocysteinemia is known to occur in preeclampsia, the cause for the elevation in preeclampsia is still not known. Further studies are needed to know the cause for hyperhomocysteinemia in preeclampsia which may help in the pharmacological management of pregnant mothers.

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PROFORMA

Name: age: Inpatient no: D.O.A:

LMP: EDD: obstetric code: D.O.D:

GESTATIONAL AGE:

COMPLAINTS OF:

Headache	Yes/no
Visual disturbance	Yes/no
Upper abdominal pain	Yes/no
Oliguria	Yes/no
Jaundice	Yes/no
Symptoms suggestive of DVT	Yes/no
Convulsions	Yes/no

HISTORY OF:

chronic hypertension	yes/no
diabetes mellitus	yes/no
chronic renal disease/hypothyroidsm	yes/no
cardio vascular disease	yes/no`
Thrombo embolism	Yes/no
Deep vein thrombosis or DVT prophylaxis	Yes/no
Repeated miscarriage	Yes/no
Neural tube defects	Yes/no
Folic acid supplementation	Yes/no
Previous h/o PIH	Yes/no

ON EXAMINATION:

Conscious: orientation: dyspnea:

Pallor: icterus: pedal edema:

Signs of DVT:

Anthropometry:

Height: weight:

Vitals:

Pulse rate: respiratory rate: Bood pressure: temperature:

Cvs: **RS:** **CNS:**

Abdomen:

Uterus size:

uterus acting: yes/no Tense/tenderness:

Fetal position: FH:

Others:

p/v:

INVESTIGATIONS:

COMPLETE HEMOGRAM:

Hb: Tc: Dc: platelet:

ESR: Hematocrit:

FBS: PPBS:

Blood urea: serum creatinine: electrolytes:

Serum uric acid: serum fibrinogen:

LFT:

Total bilirubin: ALT: AST:

SAP: Total protein: albumin:

Urine:

Albumin: sugar: deposits:

24 hours urinary protein:

Urine culture with colony count:

Peripheral smear study:

Fundus examination:

Serum homocysteine :

USG:

CTG:

DIAGNOSIS:

MATERNAL MORBIDITIES:yes/no if yes:

MATERNAL MORTALITY: yes/no

PERINATAL OUTCOME: dead/ alive

Birth weight: APGAR score: IUGR: yes/no NICU : yes/no

Neonatal mortality: yes/no term/preterm preterm: spontaneous/iatrogenic

MASTER CHART

homogr1	homogr2	homogr3	GA Gr1	GA Gr2	GA Gr3	T/PT Gr1 T/PT Gr2	T/PT Gr2	T/PT Gr3
						T	T	T
7.6	13.1	19.1	34	39	38	T	Pt(spont)	PT (iatro)
8.2	11.4	14.8	37	38	36	T	T	T
7.2	13.2	18.6	35	33	36	T	T	PT(iatro)
7.8	12.1	21.1	37	37	32	T	T	T
6.6	15.2	15.7	38	35	35	T	T	PT(iatro)
7	13.3	17.3	39	38	37	T	T	T
6.8	4.7	12	36	34	36	T	T	PT(iatro)
10.4	9	14	37	37	37	T	T	T
6.3	11	15.4	33	38	36	T	T	T
5.9	9.5	12	38	36	38	T	T	PT(iatro)
6.5	10.4	16.5	34	37	32	T	T	PT(iatro)
7.1			39	38	35	T	T	PT(iatro)
6.8	15.3	15	37	34	37	T	T	T
10	12.5	16.6	35	39	36	T	T	PT(iatro)
	15.5	15.5	38	38	36	T	T	T
7.5	9	19	34	35	37	T	T	T
7	9	13.6	39	37	36	T	T	PT(spont)
9	8.5	18.8	35	38	36	T	T	T
6.8	8.5	24	37	33	34	T	T	PT(iatro)
4.7	12.3	22.4	37	37	36	T	T	T
6.6	15.4	17.4	39	38	36	T	PT(spont)	T
5.5	13.5	13.8	35	38	38	T	T	T
4.6	12.5	19.9	38	35	37	T	T	PT(iatro)
4.3	13	22.6	39	37	35	T	T	PT(iatro)
6.4	9.5	14	35	37	35	PT(spont)	T	PT(iatro)
5.6	13	19.5	38	35	35	T	T	T
6.9	11.4		36	38	37	T	T	T
7.1		22.5	35	37	36	T	T	T
5.2	11.4	16.7	37	36	36	T	T	T
	12.2	19	34	35	35	T	T	PT(iatro)
4.9	11.7	14.7	38	32	38	T	T	T
4.6	15.3	14.2	37	36	35	T	T	T
5.8	12.7	9.5	35	37	36	T	T	T
7.1	11.9	17	39	37	36	T	T	PT(iatro)
6	12	16.8	39	35	37	T	T	PT(spont)

10.7	15.3	13.6	36	37	35	T	PT(spont)	T
4.8	9.6	15.8	37	37	37	T	T	T
4.3	9.8	14.9	38	36	35	T	T	T
4.6	9.6	9.9	38	37	36	T	T	PT(spont)

LBW Gr 1	LBW Gr2	LBW Gr3	IUGR Gr1	IUGR Gr2	IUGR Gr3	NICU Gr1	NICU Gr2	NICU Gr3
N	N	N	NO	NO	N	NA	NA	N
N	N	YES	NO	NO	YES	NA	<3 DAYS	<3DAYS
N	YES	N	NO	NO	N	NA	NA	N
N	N	YES	NO	NO	N	NA	NA	>3 DAYS
N	N	N	NO	NO	N	NA	NA	N
N	N	YES	NO	NO	N	NA	NA	>3DAYS
N	N	N	NO	NO	N	NA	NA	N
N	N	YES	NO	NO	N	NA	NA	<3DAYS
N	N	N	NO	NO	N	NA	NA	N
N	N	N	NO	NO	N	NA	NA	N
N	N	YES	NO	NO	N	NA	NA	<3 DAYS
N	N	YES	NO	NO	N	NA	NA	>3DAYS
N	N	YES	NO	NO	N	NA	NA	>3DAYS
N	N	N	NO	NO	N	NA	NA	N
YES	N	N	NO	NO	N	NA	NA	N
N	N	YES	NO	NO	N	NA	NA	N
N	N	N	NO	NO	N	NA	<3 DAYS	N
N	N	YES	NO	NO	N	NA	NA	<3 DAYS
N	N	N	NO	NO	N	NA	NA	N
N	N	YES	NO	NO	YES	NA	NA	N
N	N	YES	NO	NO	N	<3DAYS	NA	N
N	YES	N	NO	NO	N	NA	>3 DAYS	N
N	N	N	NO	NO	N	NA	NA	N
N	N	YES	NO	NO	N	NA	NA	>3 DAYS
N	N	YES	NO	NO	N	NA	NA	>3DAYS
N	N	YES	NO	NO	N	<3 DAYS	NA	<3 DAYS
N	YES	N	NO	NO	N	NA	NA	N
YES	N	YES	NO	NO	N	NA	>3 DAYS	N
N	N	N	NO	NO	N	NA	NA	N
N	N	N	NO	NO	N	NA	NA	N
N	N	N	NO	NO	N	NA	>3 DAYS	N
N	N	YES	NO	NO	N	NA	NA	N
N	N	N	NO	NO	N	NA	NA	N
N	N	N	NO	NO	N	NA	NA	N
N	YES	YES	NO	NO	N	NA	NA	<3 DAYS
N	YES	YES	NO	NO	N	NA	NA	>3DAYS

N	N	YES	NO	NO	N	NA	>3 DAYS	N
N	N	N	NO	NO	N	NA	NA	N
N	N	N	NO	NO	N	NA	NA	N
N	N	YES	NO	NO	N	NA	NA	>3 DAYS

Apgar Gr1	Apgar Gr2	Apgar Gr3	MB gr1	MBgr2	MB Gr3	Nndeath 1	NNdeath2	NNdeath3
7	8	7	NO	NO	NO	NO	NO	no
8	7	7	NO	NO	NO	NO	NO	no
9	7	7	NO	NO	NO	NO	NO	no
8	8	5	NO	NO	YES(P.edema,AKI)	NO	NO	no
6	8	NA	NO	NO	yes(ab)	NO	NO	non
7	7	7	NO	NO	no	NO	NO	no
7	8	8	NO	NO	NO	NO	NO	no
8	7	7	NO	NO	NO	NO	NO	no
7	7	3	NO	NO	NO	NO	NO	**yes**
8	7	8	NO	NO	NO	NO	NO	no
7	7	7	NO	NO	NO	NO	NO	no
8	5	6	NO	NO	NO	NO	NO	no
7	7	6	NO	NO	YES(ab)	NO	NO	no
8	8	7	NO	NO	NO	NO	NO	no
7	8	8	NO	NO	NO	NO	NO	no
8	8	8	NO	NO	NO	NO	NO	no
8	8	7	NO	NO	NO	NO	NO	no
7	8	7	NO	NO	NO	NO	NO	no
7	8	8	NO	NO	NO	NO	NO	no
7	7	8	NO	NO	NO	NO	NO	no
3	7	8	NO	NO	NO	NO	NO	no
8	7	6	NO	NO	NO	NO	NO	no
7	7	8	NO	NO	NO	NO	NO	no
7	7	4	NO	NO	YES(PPH,P edema)	NO	NO	**yes**
7	6	7	NO	NO	NO	NO	NO	no
5	6	7	NO	NO	NO	NO	NO	no
8	7	8	NO	NO	NO	NO	NO	no
7	5	7	NO	NO	NO	NO	NO	no
8	7	7	NO	NO	NO	NO	NO	no
7	8	7	NO	NO	NO	NO	NO	no
8	3	8	NO	NO	YES	NO	NO	no
7	8	9	NO	NO	NO	NO	NO	no
8	7	8	NO	NO	NO	NO	NO	no
7	8	8	NO	NO	NO	NO	NO	no
7	7	7	NO	NO	NO	NO	NO	no

7	7	5	NO	NO	YES(ab,HELLP	NO	NO	no
8	4	8	NO	NO	NO	NO	NO	no
8	8	NA	NO	NO	YES(ab,HELLP	NO	NO	no
8	7	9	NO	NO	NO	NO	NO	no
8	8	6	NO	NO	NO	NO	NO	no

IUD Gr1	IUD Gr2	IUD Gr3
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	YES
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N
NO	NO	N

NO	NO	N
NO	NO	N
NO	NO	YES
NO	NO	N
NO	NO	N